JOURNAL OF DAIRY SCIENCE

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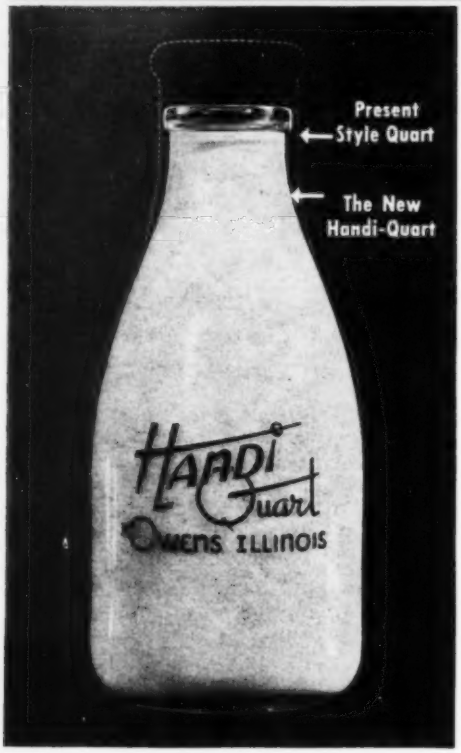

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The Journal of Dairy Science is issued monthly. Subscription is by the volume and one volume is issued per year.

Manuscripts should be typewritten and carefully revised before submission to T. S. Sutton, The Ohio State University, Columbus, Ohio. Twenty-five reprints will be furnished gratis to authors. Cost of additional reprints and reprint order blank will be submitted with proof.

The use of material published in the Journal is encouraged and a liberal policy will be followed concerning reproduction of articles with proper notation as to source.

Subscriptions. Price; $6.00 per volume in United States and Canada; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Correspondence regarding business policies of the Journal should be addressed to the Secretary-treasurer, R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

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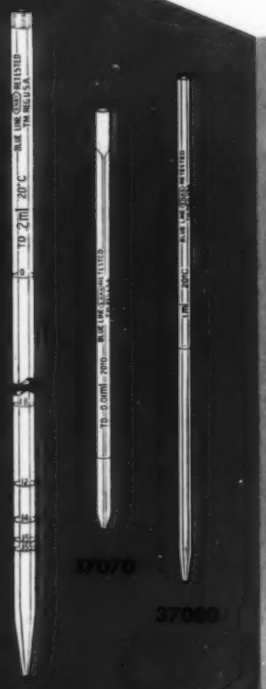

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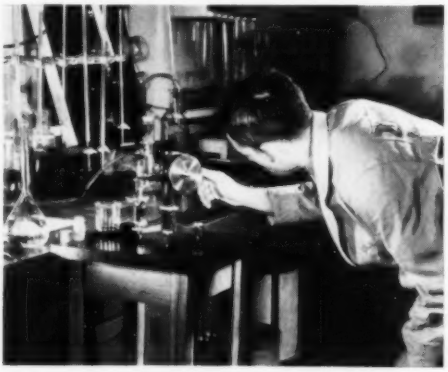

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIV MAY, 1941 NUMBER 5

STUDIES ON THE PHOSPHATASE TEST AND ITS APPLICATION TO PROCESSED AND TREATED MILKS[1]

N. A. PERRY AND F. J. DOAN

The Pennsylvania State College, State College, Pa.

The phosphatase test of Kay and Graham (1) has been modified and improved by several workers; has been used by many laboratories and health agencies supervising milk supplies; and in general has been found a very useful means of detecting imperfections and errors in the pasteurization of milk such as have been enumerated by Roger (2). A comprehensive review of the studies made of the phosphatase test and the results possible with its use, has been presented by Burgwald (3).

While a few investigations have been reported on the application of the phosphatase test to homogenized milk, preserved milk, chocolate milk, old milk and milk inoculated with various bacterial cultures, etc., (3, 4), there are a number of other practices, processes or modifications employed in the preparation and distribution of milk which might be expected to influence the application of the test. Some of these are incidental, such as contamination with metallic ions, exposure to light and development of oxidized flavor. Others are applied, such as irradiation, addition of vitamin D concentrates, homogenization and enzyme treatment. The investigation here reported was for the purpose of determining the effect of such things on the accuracy of the test. In the course of the studies variations in results were noted due to the time interval allowed for development of color following the addition of Gibbs' indicater (BQC). There seems to be some difference of opinion concerning the rapidity with which the color reaction takes place and its completeness. For this reason the matter was given some attention in an effort to evaluate its importance.

EXPERIMENTAL METHODS

Scharer's laboratory modification (5, 6) of Kay and Graham's phosphatase test was employed in this investigation because it requires less time, the reagents are not so difficult to prepare and the indicator (Gibbs' phenol

Received for publication November 28, 1940.

[1] Authorized for publication on November 22, 1940, as Paper No. 1004 in the Journal Series of The Pennsylvania Agricultural Experiment Station.

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reagent) is more specific for phenols than is Folin and Ciocalteau's reagent (3, 4, 7). Scharer's method calls for an incubation period of one hour at 95–115° F. (35–46° C.) but it has been suggested that greater sensitivity can be obtained with a two-hour period and a temperature over 100° F. (37.8° C.). In this study the samples were incubated at 105° ± 0.2° F. (40.5° C. ± 0.1° C.) for two hours. After the addition of the indicator to the test solutions, the intensity of color developed was determined at the end of exactly fifteen minutes and again after exactly thirty minutes.

In preliminary studies, a visual colorimeter was used to determine the intensity of color developed. In the investigation proper a Klett-Summerson photoelectric colorimeter (photelometer) was used with more satisfactory results than could be had with the visual colorimeter.

For some of this work, it was deemed desirable to simulate commercial conditions of pasteurization as closely as possible. In such cases, one quart samples were pasteurized in a one-liter, three-necked, round-bottom flask, fitted with an electric stirrer and an accurate thermometer. Preheating required nine to ten minutes in a separate bath held at 155–160° F. (68.8–71.0° C.) after which the flask was transferred to a constant temperature bath. It was possible to hold a sample at any given temperature, plus or minus 0.1° F. (0.05° C.). When it was necessary to remove samples at intervals, they were taken by means of a 9-ml. cream pipette, placed in test tubes and cooled in running tap water.

In other cases where it was impractical to pasteurize an entire set of samples individually in the flask and only a comparison between samples was desired, they were pasteurized in test tubes in the constant temperature bath. Preheating in such cases was also accomplished in the bath in three to five minutes.

EXPERIMENTAL RESULTS, PART I

Calibration of the Photelometer. A suitable light filter for use with the Klett-Summerson photelometer was determined by means of a Coleman spectrophotometer. The typical blue color was developed in 5 ml. of a 0.5 p.p.m. solution of phenol by the addition of 0.25 ml. of the borate buffer[2] and two drops of BQC (2,6 dibromoquinonechloroimide), allowing 30 minutes for color development. The percentage transmission of light was then measured using wave lengths at 30 millimicron intervals from 350 to 880 millimicrons. The least transmission of light (region of greatest sensitivity) was found in the band having a median wave length of 600 milimicrons. A number 66 filter (660 millimicrons) was immediately available and since this difference in wave length did not materially affect the sensitivity, it was used in this study.

A calibration curve for the purpose of converting subsequent phospha-

[2] 0.5 ml. is now recommended.

tase test readings to phenol equivalents (parts per million) was obtained for the Klett-Summerson photelometer as follows:

A series of standard phenol solutions containing from 0.1 to 10 p.p.m. of phenol were prepared following the method used by Kay and Graham (1). Color standards (5) were prepared by placing 5 ml. of the above solutions in colorimeter tubes, adding 0.25 ml. of buffer and at intervals of thirty seconds, adding 2 drops of BQC indicator to each tube in turn. Photelometer readings were made after exactly 15- and 30-minute periods of color development.

The average photelometer readings for four determinations are given in table 1 and are shown graphically in figure 1.

TABLE 1

Photelometer readings on phenol color standards

Phenol	Color development period	
	15 min.	30 min.
	Photelometer readings	
p.p.m.		
0.1	17.0	23.1
0.2	24.4	31.7
0.3	33.3	41.4
0.4	42.6	51.2
0.5	49.5	59.2
0.6	58.8	68.1
0.7	66.7	76.9
0.8	75.5	86.4
0.9	82.8	95.2
1.0	91.9	105.0
2.0	134.0	178.0
3.0	183.0	244.0
4.0	227.0	298.0
5.0	255.0	319.0
7.0	296.0	345.0
10.0	329.0	358.0

The scale of the Klett-Summerson photelometer is graduated logarithmically so that the data should lie along a straight line if the development of color is in accordance with Beer's Law. That this is true for the solutions from 0.1 to 1.0 p.p.m. of phenol may be seen in the enlarged section of figure 1. At higher concentrations of phenol, color development is less than would be expected from Beer's Law, the 15-minute color development curve deviating at a lower phenol value than the 30-minute curve.

These data indicate that color development in phenol solutions is not complete in 15 minutes, particularly at the higher phenol levels but is significantly incomplete even at the lower levels.

Influence of Time Allowed for Development of Color. Gibbs (7) has shown that the intensity of color in a phenol solution treated with BQC

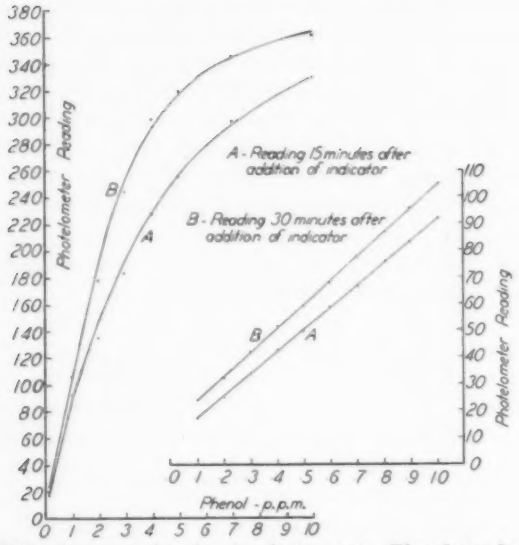
Photelometer Reading
A - Reading 15 minutes after addition of indicator
B - Reading 30 minutes after addition of indicator
Phenol - p.p.m.

FIG. 1. Calibration curve for the phosphatase test. Phenol standards, 0.1 to 10.0 p.p.m. of phenol.

indicator is a function of the time allowed for color development. Scharer's method (5, 6) stipulates a color development period of 15 minutes and it is inferred that the maximum color is developed in this length of time. A study was made of the color development in a 0.40 p.p.m. solution of phenol using a Coleman spectrophotometer. The curve obtained, plotting per cent transmittance of light against time is presented in figure 2.

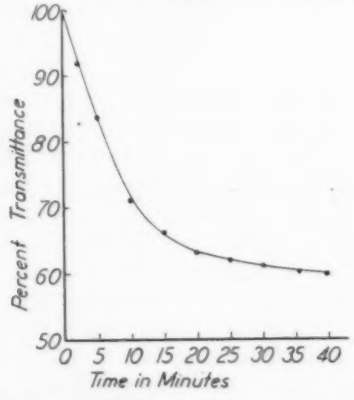

FIG. 2. Intensity of color developed in a 0.4 p.p.m. solution of phenol as affected by time allowed for development.

It may be seen that the development of color in a very dilute solution of phenol is almost complete at the end of 30 minutes but not at the end of 15 minutes. The time allowed for color development in solutions of phenol is obviously an important factor in the calibration of a photelometer for use with the phosphatase test. This was confirmed for the development of color in phosphatase tests with milk as well as with solutions of phenol.

Mixed herd milk was pasteurized in test tubes at 141°, 142°, 143°, 144° and 145° F., samples being removed after holding intervals of 20, 25 and 30 minutes, respectively. Photelometer readings were made on these samples, after applying the phosphatase test, and after allowing 15, 30, 45 and 60 minutes, respectively, for the development of color following the addition of the BQC indicator. The results of this study are shown in table 2 and the data for two of the pasteurization treatments (143° and 145° F.) are presented graphically in figure 3.

TABLE 2

Influence of color development period upon the intensity of color produced in the phosphatase test

Pasteurization		Color development period—minutes			
Temperature	Time	15	30	45	60
		Photelometer readings			
°F.	min.				
141	20	248	276	276	276
	25	147	220	228	228
	30	95	151	166	165
142	20	145	216	222	222
	25	80	129	140	143
	30	49	80	92	95
143	20	64	104	117	121
	25	39	62	72	74
	30	33	50	62	65
144	20	48	77	90	93
	25	29	46	55	57
	30	23	37	45	47
145	20	33	51	59	61
	25	25	40	47	51
	30	21	36	43	48

Where the amount of phenol, resulting from the action of the phosphatase enzyme, is small, the intensity of color increases for at least sixty minutes. More concentrated solutions require a shorter time to reach a maximum color. A comparison of the data shown in figure 2 with those presented in figure 3, furthermore, indicates that a constant color is approached in a shorter time with pure phenol solutions than where the phenol results from the action of the phosphatase enzyme of the milk. These findings further emphasize the fact that the phosphatase test must be considered

a conventional test requiring definite standardization of the procedure with respect to the time allowed for color development in the standards used for comparison as well as in the unknowns. This would be true regardless of the type of color comparator employed.

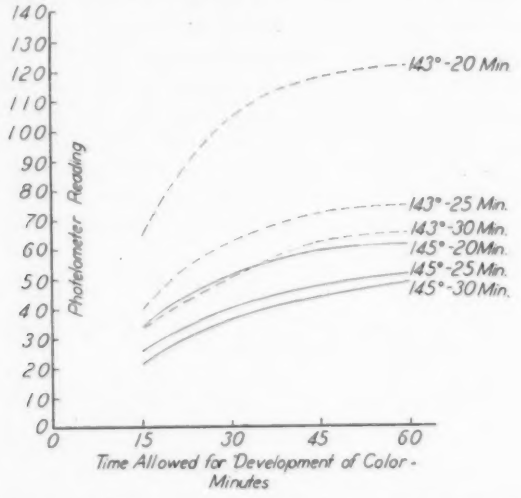

FIG. 3. Influence of time allowed for development of color upon the intensity of color produced using variously heated milk samples.

Effect of Time and Temperature of Pasteurization on Phenol Values of Milk. The practical value of the phosphatase test is dependent upon its ability to detect minor discrepancies in the pasteurization process. Other investigators (3, 5, 8, 9, 10), using Scharer's method, have generally agreed that the test is capable of detecting the following errors in pasteurization, assuming the legal standard to be 143° F. for 30 minutes: a 5 minutes shorter holding time; a lower temperature of one degree; or the addition of one-tenth of one per cent of raw milk. The same investigators, however, have not been able to agree upon the correct phenol value to set as a dividing line between properly and improperly pasteurized milk. Scharer (5) at first stated that milk pasteurized at 143° F. for 30 minutes gives a phenol value not greater than 0.5 p.p.m., but later (6) reduced this figure to 0.4 p.p.m. Other investigators have indicated Scharer's value to be too low. Tracy and Hahn (10) first set the dividing line at 0.6 p.p.m. then changed it to 0.8 p.p.m. (9). The work of Burgwald and Giberson (8) indicated 0.9 p.p.m. as the critical value.

A study was made of the progressive destruction of phosphatase by pasteurization of one-quart samples of mixed herd milk at temperatures

from 142 to 145° F. Samples were taken before pasteurization and at the end of 5, 10, 15, 20, 25 and 30 minutes of holding at the specified temperatures, ± 0.1° F. They were then cooled in ice water. The time of preheating varied from 9 to 10 minutes. The average phenol values of triplicate determinations are presented in table 3 and shown graphically in figure 4. The raw milk gave an average phenol value of 7.7 p.p.m. when 15 minutes were allowed for color development and the photelometer readings were referred to the 15-minute phenol standard. The average phenol value was 4.4 p.p.m. using a 30-minute color development period and referring the readings to the 30-minute phenol standard.

TABLE 3

Effect of pasteurization temperature and holding time upon the phenol values of milk (expressed in parts per million of phenol)

Holding time	Pasteurization temperature			
	142° F.	143° F.	144° F.	145° F.
	Color developed for 15 minutes and referred to the 15-minute phenol standard			
min.				
5	6.0	5.5	4.7	4.8
10	5.0	4.3	2.3	1.0
15	3.5	1.8	0.67	0.32
20	1.8	0.65	0.25	0.15
25	0.85	0.31	0.13	0.11
30	0.51	0.18	0.09	0.10
	Color developed for 30 minutes and referred to the 30-minute phenol standard			
5	3.5	3.3	3.0	3.0
10	2.7	2.8	2.0	1.2
15	2.4	1.8	0.82	0.40
20	1.3	0.85	0.34	0.20
25	1.0	0.43	0.18	0.16
30	0.61	0.28	0.12	0.15
	Average phenol value of the raw milk was 7.7 p.p.m. with a 15-minute period for color development and 4.4 p.p.m. with a 30-minute period.			

These data demonstrate that the phosphatase test is more accurate in detecting discrepancies in pasteurization with the lower than with the higher temperatures. It is possible to detect underheating of 5 minutes at 142 or 143° F., of 10 minutes at 144° F. and of 15 minutes at 145° F. It is also possible to detect too low a temperature of one degree when the standard is 143 or 144° F. and of two degrees when the standard is 145° F. The results further indicate that the following heat treatments of milk are equally destructive of phosphatase: 143° F. for 30 minutes; 144° F. for 23 minutes; and 145° F. for 19 minutes.

A comparison of the upper and lower parts of table 3, or the two sets of curves in figure 4, reveals a very definite difference in phenol values obtained

using 15 minutes for color development as compared with 30 minutes. Where the amounts of phenol present are relatively large (over 1.5 p.p.m.) the 15-minute period of color development produces higher phenol values than the 30-minute period, whereas the opposite is true when the amounts of phenol are relatively small. From a practical standpoint, either procedure may be used with only a slight shift in the phenol value representative of proper pasteurization but tests made without careful control of the period allowed for color development cannot be depended upon.

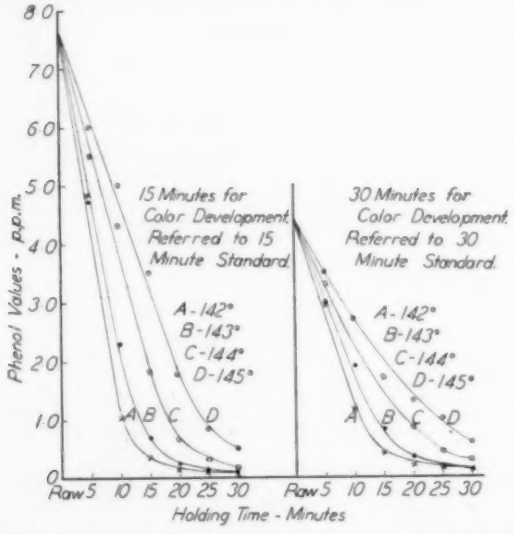

FIG. 4. Effect of pasteurization temperature and holding time upon the phenol value of milk.

The practical use of the phosphatase test necessitates the designation of an arbitrary value as the dividing line between properly and improperly pasteurized milk. This study indicates that milk pasteurized at 143° F. for 30 minutes has a phenol value of less than 0.25 p.p.m. when a 15-minute color development period is used and the photelometer readings are referred to the 15-minute phenol standard. However, when a 30-minute period is used and the readings are referred to the 30-minute phenol standard, the same milk has a phenol value of somewhat less than 0.40 p.p.m. This latter procedure is, of course, a digression from Scharer's published method and was used only to illustrate the importance of the length of time allowed for color development.

It is difficult to adequately explain the difference of opinion among the various investigators concerning the correct phenol value representative of

proper pasteurization. No investigator has mentioned the importance of the length of time allowed for color development in the color standards and in the phosphatase test and it is believed that variations in this factor may be largely responsible for the dissimilarity of the results obtained. Burgwald and Giberson (8) suggest that variations in sensitivity of the BQC indicator may be a factor. Another contributing cause may be the different methods of standardizing the phenol solutions and of preparing the phenol color standards.

Effect of the Addition of Raw Milk to Pasteurized Milk on the Phenol Value. Studies were made as to the effect of additions of small quantities of raw milk to milk properly pasteurized at 142° F. to 145° F. on the phenol values obtained with the phosphatase test. The results indicated that quantities of raw milk as small as 0.1 per cent can be definitely detected where the temperature of pasteurization is known, increases in the phenol value for a given amount of raw milk being relatively uniform regardless of the pasteurization temperature. Detection is more certain using a 15-minute period for color development since the phenol values are lower.

EXPERIMENTAL RESULTS, PART II

In the following studies the photelometer readings were made on all samples after a 15-minute period for color development and were referred to the 15-minute phenol standard for conversion into p.p.m. of phenol.

Influence of Fortification of Milk with Vitamin D on the Phosphatase Test. No information is available in the literature to indicate whether the phosphatase test is applicable to vitamin D reinforced milks. While it would probably not be expected that the addition of vitamin D concentrates would have any effect upon the accuracy of the test, it is conceivable that the activity of the phosphatase enzyme might be affected by the treatment of milk with ultraviolet radiations. Studies were made with irradiated milk and with milk reinforced with Vitex, Clo-Dee and A.R.P.I. vitamin D concentrates.

Irradiation. Two series of commercially pasteurized milk samples were obtained from different sources. Each consisted of three sets of samples from different lots of milk, one portion being irradiated subsequent to pasteurization; the other portion, unirradiated. The treatment of the samples and the resulting phenol values of phosphatase tests are presented in table 4.

Vitamin D Concentrates. Other series of samples were prepared in the laboratory from mixed herd milk using vitamin D concentrates and were pasteurized at 143° F. for 30 minutes. Samples were made up to contain 400 and 800 International Units per quart from four concentrates, Vitex, Clo-Dee, A.R.P.I. No. 6 and A.R.P.I. No. 15. The phenol values obtained in phosphatase tests are shown in table 5.

The data indicate rather conclusively that milk may be reinforced with

TABLE 4

Influence of irradiation on the phenol values of milk (expressed in p.p.m. of phenol)

Samples	145° F.—30 min.*		160° F.—16 sec.*		143° F.—30 min.†	
	Not irradiated	Irradiated 135 I.U. Vit. D	Not irradiated	Irradiated 135 I.U. Vit. D	Not irradiated	Irradiated 400 I.U. Vit. D
1st set	0.11	0.13	0.09	0.07	0.11	0.105
2nd set	0.10	0.10	0.10	0.10	0.10	0.105
3rd set	0.14	0.15	0.13	0.13	0.10	0.09

* Samples made available through the courtesy of F. M. Greenleaf of the Lehigh Valley Cooperative Farmers Association, Allentown, Pa.

† Samples obtained through the cooperation of Dr. K. G. Weckel, Department of Dairy Industry, University of Wisconsin.

TABLE 5

Influence of adding vitamin D concentrates before pasteurization on the phenol values of milk

Samples	Vitex	Clo-Dee	A.R.P.I. No. 6	A.R.P.I. No. 15
Control—No concentrate	0.105	0.125	0.125	0.13
400 I.U. per quart	0.115	0.14	0.13	0.13
800 I.U. per quart	0.09	0.11	0.13	0.15

vitamin D by irradiation or by the addition of vitamin D concentrates without affecting the accuracy of the phosphatase test.

Influence of Pancreatic Enzymes Added to Milk before Pasteurization on the Phosphatase Test. The addition of a pancreatic extract has been proposed (11) as a means of lowering the curd tension of milk. The extract is available under the name Enzylac and is used by introducing a very small amount into milk, allowing a short period of time for enzymatic action and then immediately pasteurizing. The pasteurization destroys the enzymes and no further action takes place. The use of this extract in a similar manner at the rate of one gram of extract to each ten to forty gallons of milk, has also been suggested (12) as an effective means of preventing oxidized flavor. This practice is illegal in most states but is being used in some places with or without the sanction of the health agencies.

To determine whether the presence or the action of the enzymes might have an effect upon the phosphatase test, a series of samples was prepared from mixed herd milk containing the recommended concentration of enzyme and pasteurized at 143° ± 0.1° F. Table 6 shows the phenol values obtained in phosphatase tests from these samples.

It is apparent that the addition of pancreatic extract (Enzylac) to milk before pasteurization in amounts necessary to produce soft curd milk or to prevent oxidized flavor has no effect upon the phosphatase test.

Influence of Contamination of Milk with Small Quantities of Metallic Ions on the Phosphatase Test. During the handling and pasteurization

TABLE 6

Influence of additions of pancreatic enzyme to milk before pasteurization on the phenol values

Concentration of enzyme	Phenol
	p.p.m.
None	0.13
1 gram/40 gal.	0.14
1 gram/10 gal.	0.14

process, milk necessarily picks up traces of metallic ions and holds them in solution. Since enzymes are known to be activated by certain ions and inhibited by others, a study was made to ascertain what effect, if any, the following ions have on the phosphatase test: copper, nickel, iron, aluminum and tin. The ions were added to the mixed herd milk in concentrations up to ten parts per million and the samples were pasteurized in test tubes at 143° F. for 30 minutes. The average phenol values of duplicate phosphatase determinations, are presented in table 7.

TABLE 7

Phenol values of milk contaminated with metallic ions before pasteurization (expressed in p.p.m. of phenol)

Concentration of ion	Ions added				
	Cu	Ni	Fe	Al	Sn
p.p.m.					
0	0.12	0.15	0.12	0.15	0.13
0.5	0.11	0.13	0.12	0.13	0.16
1.0	0.12	0.15	0.11	0.17	0.17
3.0	0.13	0.12	0.10	0.14	0.17
5.0	0.12	0.12	0.10	0.16	0.17
7.0	0.11	0.10	0.09	0.15	0.13
10.0	0.11	0.11	0.11	0.13	0.13

Similar results were obtained with samples which were contaminated with these ions after pasteurization. Apparently metallic contamination such as might occur in the normal handling of milk exercises a negligible effect on the phosphatase test.

Influence of the Development of Oxidized Flavor in Milk on the Phosphatase Test. A one quart sample of mixed herd milk was pasteurized at 143° ± 0.1° F. for 30 minutes and divided into three parts. The first portion was used as a control; copper was added to the second at the rate of two p.p.m.; the third was exposed to bright sunlight for one hour. The sample containing copper was oxidized at the end of 24 hours and became progressively worse. The sunlight sample developed a typical burnt or activated flavor. The samples were tested immediately and at the end of 24, 48, and 72 hours storage at 40° F. Table 8 shows the average phenol values of triplicate phosphatase determinations.

TABLE 8

Influence of the development of oxidized flavor in milk on the phenol value (Expressed in p.p.m. of phenol)

Samples	Storage period			
	Immediately	24 hrs.	48 hrs.	72 hrs.
Control	0.10	0.14	0.15	0.16
Cu. 2 p.p.m.	0.10	0.13	0.16	0.16
Sunlight	0.10	0.12	0.14	0.15

These data indicate no significant difference between the control and the treated samples. While there is a more or less regular increase in phenol value for all of the samples, it is too slight to be significant and may be due to experimental variations.

Other Studies. Homogenization of milk was found to have no influence on the phosphatase test as has been noted by other investigators (3, 9, 13). Studies on milk held for periods up to six days under refrigeration indicate that, as others have shown (5, 8, 14, 15, 16), the age of milk does not affect the accuracy with which the phosphatase test may be applied. Data for these studies are omitted.

CONCLUSIONS

1. A photoelectric colorimeter (photelometer) offers a convenient and accurate means of determining the intensity of color produced by the phosphatase test. It has the advantage of permitting an exact period of time for color development, and phenol standards, which are unstable, need be prepared only once.

2. The proper filter to use with Scharer's modification of the phosphatase test, if color intensities are to be determined with a photelometer, is one which will exclude practically all of the light except that in a rather narrow band of the spectrum having a median wave length of approximately 600 millimicrons.

3. The development of color in dilute solutions of phenol, using 2,6,-dibromoquinonechloroimide (BQC) as an indicator, is practically complete at the end of thirty minutes but not at the end of fifteen minutes. Therefore, in the calibration of a photelometer, the period of time allowed for color development in the standard phenol solutions should be carefully controlled.

4. Phosphatase tests made on milk samples containing relatively small quantities of active phosphatase enzyme, increase in color intensity for at least sixty minutes. More concentrated solutions require a shorter time to reach a maximum color. It is essential to allow a uniform carefully controlled period of time for color development in the performance of the phosphatase test and to make photelometer readings at exact intervals.

5. The phosphatase test is capable of detecting underheating of 5 minutes at 142 and 143° F., of 10 minutes at 144° F., and of 15 minutes at 145° F.

It is capable of detecting too low a temperature of one degree when the pasteurization standard is 143 or 144° F. and of two degrees when the standard is 145° F. As little as one-tenth of one per cent of raw milk may be detected when added to milk which is assumed to have been pasteurized at any specific temperature between 142 and 145° F. for 30 minutes.

6. The accuracy of the phosphatase test as applied to pasteurized milk is not affected to a significant extent by any of the following treatments or processes:

a. Reinforcement of milk with vitamin D either by irradiation or by the addition of vitamin D concentrates;
b. Addition of pancreatic extract (Enzylac) for producing a soft curd type of milk or for preventing oxidized flavor;
c. Contamination with such metallic ions as copper, nickel, iron, aluminum, or tin in concentrations up to ten parts per million;
d. Development of oxidized flavor;
e. Homogenization;
f. Storage under refrigeration for periods as long as six days.

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MASTITIS. I. THE RELATIONSHIP OF THE DEVELOPMENT OF MASTITIS TO CHANGES IN THE CHLORINE, LACTOSE AND CASEIN NUMBER OF MILK*

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The secretion of milk of abnormal composition by cows with latent or chronic mastitis has been generally recognized for a long time. In 1937 Hastings and Beach (4) summarized the prevailing opinion that approximately ninety per cent of the cases of chronic mastitis are due to *Streptococcus agalactiae* and the remainder to other bacteria such as various species of *Streptococcus, Staphylococcus, Micrococcus* and varieties of *Escherichia coli.*

The most apparent chemical changes in milk due to mastitis or associated with mastitis are decreases in the fat, solids-not-fat, casein, lactose, ash and titratable acidity, and increases in water, non-casein nitrogen, chlorine and pH value. Certain of these chemical constituents have been determined in milk from diseased udders and compared with milk from non-infected udders as an indirect biochemical method for detecting mastitis. The chemical constituents most commonly determined for this purpose are the chlorine, the lactose, and the pH value of the milk. Normal milk is said to contain less than 0.14 per cent chlorine, at least 4 per cent lactose, and to have a pH value below 6.8 (4, 6).

Factors other than mastitis such as an increase in the number of lactations, the stage of lactation and a decrease in the solids-not-fat content of the milk are known to cause an increase in the percentage of chlorine in the milk (3, 5, 8, 15). There is also evidence that milk from udders free of mastitis may at times contain more than 0.14 per cent chlorine (3, 4, 5, 10). Hastings and Beach (4) have also demonstrated wide differences in the chlorine, acidity, catalase and pH values of milk depending upon whether the sample was fore-milk or whole milk. Johns and Hastings (7) observed a marked fluctuation, often of a rhythmic character, in the chlorine, catalase and pH value for fore-milk from milking to milking. Infected quarters as shown by the presence of *Streptococcus* were found frequently to yield normal milk while non-infected quarters yielded milk giving definite abnormal reactions. These investigators stressed the need of caution in the use of indirect tests as the basis for diagnosis of mastitis and emphasized

Received for publication December 9, 1940.

* Published with the approval of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper No. 250.

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the value of examining a series of samples at consecutive milkings in order to obtain a true picture of the condition of the quarter. Davis (3) found that the percentage of chlorine was very uniform in the milk of cows in good health, whether the period of sampling was monthly, weekly, or daily, but milk high in chlorine showed considerable variation when sampled daily or weekly.

Koestler (9) proposed the chlorine-lactose number

$$\left(\frac{\text{per cent chlorine}}{\text{per cent lactose}} \times 100\right)$$

for detecting mastitis. In cases of mastitis the chlorine increases whereas the lactose decreases, therefore the chlorine-lactose number changes proportionately more than either chlorine or lactose alone.

Rowland (12) made an extensive study of the protein distribution in normal and abnormal milks, and found that cows with sub-clinical mastitis can be detected by determining the "casein number" which is the percentage casein nitrogen of the total nitrogen in the milk.

Rogers and associates (2) reviewed a limited number of studies made on the composition of milk from individual quarters of the same udder. The results indicate wide differences in the chemical composition of milk from healthy and diseased quarters of the same udder.

Since various uncontrollable factors other than the health of the udder are known to affect the chemical composition of milk, it seemed desirable to make a study of the composition of milk from individual quarters of the same udder for entire lactation periods. By studying several constituents in the milk, such as the chlorine, lactose and nitrogen distribution or "casein number," it was thought that possibly one constituent might show a significant change at an earlier stage in the development of mastitis than the others, thus making it possible to increase the accuracy of an indirect biochemical test for the diagnosis of mastitis.

EXPERIMENTAL

This investigation was started in the fall of 1938 in connection with a study of the possible relationship of certain constituents in the ration and the method of feeding as predisposing factors to the development of mastitis. When the investigation was started, twenty-two pure bred Holstein cows were available for observation. Of the twenty-two cows, seven were in the latter part of the first lactation, six were in the second lactation, two in the third, five in the fourth, one was in the fifth and one in the sixth lactation. During the course of the study eighteen pure-bred Holstein heifers in their first lactation were added to the herd making a total of forty animals under observation.

For several months preceding the present study and throughout the investigation, routine physical examinations of the udder and the following

diagnostic tests were made at biweekly intervals on samples of fore-milk; strip cup, brom-thymol blue, chlorine (colorimetric), Hotis test, leucocyte count, microscopic examination, and blood agar plate.

The diagnosis of mastitis was based upon the presence of hemolytic streptococci accompanied by changes indicated by one or more of the other tests, which supported the bacteriological findings. The presence of occasional hemolytic streptococci, or other organisms particularly hemolytic staphylococci, as well as non-hemolytic streptococci and staphylococci in large numbers, accompanied by changes indicated by the other tests that show evidence of mastitis, was considered as suspicious. If tests other than the blood agar plate showed an increase in the severity of reaction, such animals were considered highly suspicious and usually on subsequent tests were found positive.

When this investigation was started seven of the twenty-two cows were diagnosed as having latent or chronic mastitis in one or more quarters. During the period covered by this study several additional animals were diagnosed as positive or suspicious with regard to mastitis.

Samples of about six to eight ounces of fore-milk were collected at monthly intervals from each quarter at the morning milking. The samples were taken to the laboratory and analyzed for chlorine, lactose, total nitrogen and non-casein nitrogen on the same day that the samples were collected. Total nitrogen and non-casein nitrogen were determined and the casein number was calculated as described by Rowland (11, 12), except that larger samples were used and the determinations were made using regular macro Kjeldahl apparatus. Chlorine was determined according to the method of Sanders (14), except that 10 ml. of the sample with the required amount of the silver nitrate solution were diluted to 100 ml. in a volumetric flask and filtered. The excess silver nitrate was titrated in 25-ml. aliquots of the filtrate. Lactose was determined by the A.O.A.C. optical method (1), and the chlorine-lactose number was calculated as was outlined by Koestler (9).

RESULTS

In connection with this study about 250 udder examinations were made by chemical analyses of milk from individual quarters. The individual quarters were divided into three groups according to the physical condition of the quarter and the reaction of the various diagnostic tests made during the month in which the samples of milk were taken for chemical analysis. Group I included quarter samples from udders in which all quarters were negative. Group II included samples from negative quarters from udders in which one or more quarters were positive. Group III included samples from positive quarters.

A summary of the data obtained for the three groups is presented in table 1.

TABLE 1

The chlorine, lactose, chlorine-lactose number, and casein number for the individual quarters in the various groups

Diagnosis of quarters	No. of animals	No. of quarter samples	Minimum	Maximum	Mean ± S.E.	Coeff. of Var.
			Per cent chlorine			
Group I. All quarters negative in udder	26	376	0.090	0.224	0.124 ± 0.001	8.06
Group II. Neg. quarters from pos. udders	15	142	0.089	0.239	0.119 ± 0.002	18.49
Group III. Pos. quarters from pos. udders	18	201	0.087	0.273	0.150 ± 0.003	26.00
			Per cent lactose			
Group I. All quarters negative in udder	18	296	3.30	5.40	4.788 ± 0.007	2.36
Group II. Neg. quarters from pos. udders	14	121	4.00	5.75	4.872 ± 0.033	7.51
Group III. Pos. quarters from pos. udders	17	146	2.30	5.40	4.347 ± 0.065	18.15
			Chlorine-lactose number			
Group I. All quarters negative in udder	18	296	1.62	6.79	2.614 ± 0.027	17.71
Group II. Neg. quarters from pos. udders	14	121	1.67	4.70	2.457 ± 0.057	25.36
Group III. Pos. quarters from pos. udders	17	146	1.83	10.95	3.665 ± 0.149	49.25
			Casein number			
Group I. All quarters negative in udder	26	316	69.4	84.3	77.57 ± 0.11	2.56
Group II. Neg. quarters from pos. udders	15	137	68.6	83.1	77.76 ± 0.22	3.24
Group III. Pos. quarters from pos. udders	18	198	56.8	84.1	74.15 ± 0.32	6.08

From a study of the data presented in table 1 it may be seen that the minimum and maximum values obtained for the various groups are on the whole very similar. This illustrates the difficulty in the diagnosis of mastitis by changes in the chlorine, lactose, chlorine-lactose number or casein number of the milk when some arbitrary value is used as a criterion above or below which is taken as indicating mastitis. The means for the negative quarters are very similar even though one or more quarters in the same udder may have been diagnosed as positive but are significantly different from the means for the positive quarters. The means for the positive quarters show the effect of abnormal milk in this group, as the percentage chlorine was higher and the percentage lactose was lower than for the negative quarters. The means for the chlorine-lactose number was higher and the casein number lower for the positive quarters than the means for the

negative quarters. There was a successive increase in the coefficient of variation from the first to the third group. The chlorine-lactose number was subject to the greatest variation of any of the constituents studied. The percentage chlorine shows the next greatest variation, followed by the percentage lactose with the casein number showing the least amount of variation.

From physical, bacteriological and chemical evidence it is known that mastitis does not develop in the four quarters of an udder simultaneously, but generally is first observed in a single quarter and then may spread to other quarters of the udder. The milk from unaffected quarters usually appears normal in composition. If it is assumed that the milk from unaffected quarters is normal in composition, then by a comparison of the chemical composition of the milk from the individual quarters of the same udder it should be possible to eliminate to a considerable extent the effect of variation in the composition of milk due to uncontrollable factors such as season, number and stage of lactation as well as the individuality of the animal, thus making the diagnosis of mastitis by changes in the chemical composition of the milk much more accurate. In order to determine the extent of variation which might be expected among quarters of udders that were free of mastitis a study was made of the differences between quarters numbers 1[4] and 2, 1 and 3, 1 and 4, 2 and 3, 2 and 4, and 3 and 4 for all udders regardless of number and stage of lactation. For the study the following constituents were considered: chlorine, lactose, chlorine-lactose number, and casein number. In table 2 is presented, in summary form, the number of normal udder examinations, the number of animals involved, and the number of differences between quarters upon which the means and standard deviations are based. The differences between quarters were considered as individual variates in a normal distribution. The means and standard deviations were determined as usual. For a single constituent in the milk of one quarter to be considered significantly different from that of any other

TABLE 2

Number of normal animals examined, number of udder examinations, number of quarter differences—mean of differences, standard deviation and greatest difference permissible without being a significant difference between quarters

Constituent	No. of animals	No. of normal udder examinations	No. of quarter differences	Mean of differences	S. Dev.	Mean plus 2 S. Dev. (Odds 1: 19)
Chlorine	26	80	480	0.0065	0.0066	0.020
Lactose	18	74	444	0.1154	0.1234	0.362
Chlorine-lactose No.	18	74	444	0.1842	0.2081	0.600
Casein No.	26	79	474	0.9987	0.9512	2.901

[4] R.F. quarter No. 1, R.R. quarter No. 2, L.R. quarter No. 3, L.F. quarter No. 4.

TABLE 3

Variation from milking to milking for chlorine, lactose, and chlorine-lactose number for one normal animal and three animals with chronic mastitis

No. of animal	Quarter	Time of sampling					
		First day		Second day		Third day	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
		Per cent chlorine					
	1	0.108	0.116	0.102	0.106	0.105	0.110
	2	0.120	0.124	0.103	0.120	0.109	0.118
	3	0.108	0.110	0.101	0.107	0.101	0.106
	4	0.106	0.110	0.106	0.106	0.104	0.106
		Per cent lactose					
	1	5.25	5.15	5.10	5.25	5.20	5.15
475	2	5.10	4.95	5.10	5.05	5.15	5.05
	3	5.25	5.05	5.20	5.20	5.25	5.15
	4	5.25	5.20	5.20	5.15	5.30	5.15
		Chlorine-lactose number					
	1	2.06	2.25	2.20	2.02	2.02	2.14
	2	2.35	2.51	2.02	2.38	2.12	2.34
	3	2.06	2.18	1.94	2.06	1.92	2.06
	4	2.02	2.12	2.04	2.06	1.96	2.06
		Per cent chlorine					
	1	0.178*	0.166*	0.175*	0.174*	0.183*	0.168*
	2	0.131	0.139	0.136	0.140	0.138*	0.122
	3	0.162*	0.150*	0.146*	0.150*	0.146*	0.136
	4	0.124	0.122	0.122	0.122	0.118	0.122
		Per cent lactose					
	1	3.90*	3.60*	3.65*	3.95*	3.75*	3.90*
469	2	4.80	4.55	4.75	4.50	4.60*	4.80
	3	4.05*	4.30*	4.35*	4.35*	4.35*	4.45
	4	4.95	4.75	4.80	4.80	5.00	4.65
		Chlorine-lactose number					
	1	4.56*	4.61*	4.79*	4.41*	4.88*	4.31*
	2	2.73	3.05	2.86	3.11	3.00*	2.54
	3	4.00*	3.49*	3.36*	3.45*	3.36*	3.06
	4	2.51	2.57	2.54	2.54	2.36	2.62
		Per cent chlorine					
	1	0.132*	0.135*	0.125	0.162*	0.138*	0.154*
	2	0.115	0.117	0.114	0.120	0.117	0.161*
	3	0.110	0.115	0.111	0.116	0.113	0.136
	4	0.116	0.114	0.113	0.125	0.117	0.130
		Per cent lactose					
	1	4.80	4.75	4.70	4.25*	4.65*	4.65
486	2	5.15	5.10	4.90	5.00	5.10	4.75
	3	5.15	4.95	5.00	5.10	5.20	4.90
	4	5.10	4.80	4.85	4.85	4.95	4.85
		Chlorine-lactose number					
	1	2.75*	2.84	2.66	3.81*	2.97*	3.31*
	2	2.23	2.29	2.33	2.40	2.29	3.39*
	3	2.14	2.32	2.22	2.27	2.17	2.78
	4	2.27	2.38	2.33	2.58	2.36	2.68

TABLE 3.—(*Continued*)

No. of animal	Quarter	Time of sampling					
		First day		Second day		Third day	
		A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
		Per cent chlorine					
	1	0.152*	0.162*	0.158*	0.152	0.158*	0.136
	2	0.160*	0.160*	0.152*	0.173*	0.178*	0.134
	3	0.136	0.135	0.131	0.141	0.133	0.119
	4	0.124	0.130	0.127	0.133	0.124	0.122
		Per cent lactose					
	1	4.35	4.30	4.25	4.45	4.40*	4.40
490	2	4.30	4.25	4.15	4.10*	4.05*	4.20*
	3	4.55	4.55	4.50	4.40	4.70	4.60
	4	4.70	4.60	4.70	4.65	4.90	4.70
		Chlorine-lactose number					
	1	3.49*	3.77*	3.72*	3.42	3.59*	3.09
	2	3.72*	3.76*	3.66*	4.22*	4.39*	3.19*
	3	2.99	2.97	2.91	3.20	2.83	2.59
	4	2.64	2.83	2.70	2.86	2.53	2.60

* Abnormal chemical composition.

quarter with which it might be compared, the difference must be equal to or greater than the mean of the quarter differences plus two times the standard deviation.

From the data presented in table 2 it may be seen that for one quarter to be considered significantly different from any other quarter in the same udder with which it might be compared, there must be a difference of as much as 0.02 per cent in chlorine, and 0.36 per cent in lactose. There must also be a difference of 0.60 in the chlorine-lactose number and a difference of 2.90 in the casein number. These differences include variations in sampling and in analytical methods, as well as normal variations in the composition of milk from udders free from disease. For these differences the odds are 1 to 19, which allows a difference as great as these in 5 per cent of the cases and still be within the normal range of variation. The odds increase very rapidly when the differences between quarters exceed to an appreciable extent the limits considered as significant. In order to detect significant changes in the chlorine, lactose, chlorine-lactose number, or casein number of the milk due to or associated with mastitis by a comparison of quarter differences, the quarter with the lowest percentage chlorine and chlorine-lactose number, or highest percentage lactose and casein number is considered normal.

A study of the effect of mastitis on the composition of the milk from milking to milking was made by a comparison of quarter differences when samples of milk were taken at consecutive milkings, morning and evening, for a period of three days. Chlorine and lactose were determined, and the

chlorine-lactose number was calculated. Data showing variation from milking to milking are shown in table 3.

The data as shown in table 3 represent one normal animal and three animals showing evidence of chronic mastitis. Number 475 was an animal definitely negative as shown by the various diagnostic tests. During the three-day period all samples were normal. In the case of cow number 469, quarters number 1 and 3 were definitely positive. All samples taken from these quarters were definitely abnormal as shown by the percentage chlorine and lactose as well as the chlorine-lactose number with the exception of samples taken from the third quarter on the last day. The second quarter at the morning milking also showed a positive difference for that time only. Animals number 486 and 490 are border-line cases. Cow number 486 was positive for chlorine in five out of six samples taken from quarter number 1, whereas the lactose was positive only for two samples, and the chlorine-lactose number was positive in four out of the six samples. Quarter number 2 was positive for one sample only for chlorine and chlorine-lactose number, and that was for the evening sample the last day. Cow number 490 gave milk from quarters 1 and 2 which was abnormal for chlorine. The chlorine-lactose number showed positive differences much of the time but the lactose did not show significant difference except for the three last samples taken from the second quarter. Quarter number 1 also showed a positive difference on the morning of the last day.

In table 4 are presented data for four cows which show the relationship between the development of chronic mastitis as determined by diagnostic tests and changes in the chlorine, lactose, chlorine-lactose number and casein number of the milk. The data for the four animals presented in table 4 are representative of the data as a whole.

In the case of cow 424 in table 4, only one abnormal sample was obtained from the third quarter and that was during the second month of lactation; nevertheless, this quarter was diagnosed suspicious for the first month and positive throughout the remainder of the lactation period. Quarters 1 and 2 were also diagnosed positive during the latter part of the period, but only the second quarter secreted abnormal milk. Cow 416 was diagnosed negative for mastitis during the latter part of the second lactation, but an abnormal sample was obtained from quarter number 1 during the eleventh month or the last month of the second lactation period. During the first three months of the third lactation all quarters were negative, but were either positive or suspicious for the fourth and fifth months. The second and fourth quarters secreted abnormal milk during the fifth month of lactation while the first and third quarters secreted normal milk even though mastitis was definitely diagnosed in these quarters.

Cow number 373 was diagnosed negative in all quarters until the eleventh month of the fourth lactation, at which time the fourth quarter

TABLE 4

Relationship of the development of chronic mastitis to changes in the chlorine, lactose, chlorine-lactose number and casein number of the milk

Cow #424, Second Lactation

	Number of quarters	Months of lactation							
		1	2	3	4	5	6	7	8
Diagnosis	1	–	–	S	S	S	+	+	+
	2	–	–	S	+	+	+	+	+
	3	S	+	+	+	+	+	+	+
	4	–	–	–	–	–	–	S	S
Chlorine	1	0.090	0.103	0.090	0.106	0.089	0.099	0.128	0.126
	2	0.091	0.107	0.095	0.103	0.097	0.125*	0.110	0.178*
	3	0.102	0.149*	0.107	0.107	0.108	0.104	0.112	0.120
	4	0.092	0.102	0.092	0.096	0.091	0.097	0.096	0.112
Lactose	1	4.70	4.75	5.20	5.25	5.10	5.15	4.80	4.85
	2	4.70	4.60	5.35	5.25	5.00	4.65*	4.75	3.50*
	3	4.50	3.65*	5.20	5.15	5.10	5.00	4.80	4.90
	4	4.65	4.55	5.75	5.40	5.45	5.25	5.05	5.15
Chlorine-lactose number	1	1.91	2.17	1.73	2.02	1.75	1.92	2.67	2.60
	2	1.94	2.33	1.78	1.96	1.94	2.69*	2.32	5.09*
	3	2.26	4.08*	2.06	2.08	2.18	2.08	2.33	2.45
	4	1.98	2.24	1.60	1.78	1.67	1.85	1.90	2.17
Casein number	1	77.8	77.6	78.1	77.3	75.3	76.9	76.5	79.2
	2	77.4	77.0	76.5	77.2	74.6	73.8*	75.0	59.7*
	3	76.0	71.7*	75.7	76.6	73.3	76.5	76.4	81.6
	4	77.8	76.8	78.1	78.3	75.8	77.3	77.5	77.6

TABLE 4.—(*Continued*)

Cow #416, Second and Third Lactations

	Number of quarters	Months of lactation									
		9	10	11	Dry	Dry	1	2	3	4	5
Diagnosis	1	–	–	–	.....	.....	–	–	–	+	+
	2	–	–	–	.....	.....	–	–	–	+	+
	3	–	–	–	.....	.....	–	–	–	+	S
	4	–	–	–	.....	.....	–	–	–	+	+
Chlorine	1	0.128	0.146	0.171*	.....	.....	0.093	0.112	0.099	0.110	0.112
	2	0.124	0.127	0.146	.....	.....	0.097	0.111	0.096	0.110	0.175*
	3	0.129	0.140	0.141	.....	.....	0.092	0.112	0.101	0.108	0.109
	4	0.127	0.140	0.141	.....	.....	0.091	0.111	0.099	0.109	0.175*
Lactose	1	.....	.....	3.65*	.....	.....	5.35	5.05	5.15	5.05	5.10
	2	.....	.....	4.15	.....	.....	5.25	5.00	5.20	5.05	3.45*
	3	.....	.....	4.00	.....	.....	5.25	5.00	5.15	5.15	5.10
	4	.....	.....	4.05	.....	.....	4.45*	5.00	5.05	5.15	3.60*
Chlorine-lactose number	1	.....	.....	4.68*	.....	.....	1.74	2.22	1.92	2.18	2.20
	2	.....	.....	3.52	.....	.....	1.85	2.22	1.85	2.18	5.07*
	3	.....	.....	3.53	.....	.....	1.75	2.24	1.96	2.10	2.14
	4	.....	.....	3.48	.....	.....	2.04	2.22	1.96	2.10	4.86*
Casein number	1	78.6	77.6	74.9*	.....	.....	77.4	75.5	75.7	76.8	74.1
	2	79.9	77.6	77.8	.....	.....	77.4	76.3	76.6	76.5	60.2*
	3	77.1	76.7	77.6	.....	.....	77.3	75.5	71.8*	76.4	78.1
	4	77.6	77.4	76.7	.....	.....	76.4	75.5	76.6	76.5	73.9*

TABLE 4.—(*Continued*)

Cow #373, Fourth and Fifth Lactations

	Number of quarters	Months of lactation									
		9	10	11	12	Dry	Dry	Dry	1	2	3
Diagnosis	1	–	–	–	S	.....	.....	.....	S	S	S
	2	–	–	–	+	.....	.....	.....	+	+	+
	3	–	–	–	S	.....	.....	.....	S	S	S
	4	–	–	S	S	.....	.....	.....	S	S	S
Chlorine	1	0.142	0.145	0.171*	0.174*	.....	.....	.....	0.115	0.119	0.112
	2	0.139	0.146	0.161*	0.137	.....	.....	.....	0.135*	0.155*	0.252*
	3	0.128	0.131	0.139	0.128	.....	.....	.....	0.111	0.118	0.102
	4	0.138	0.146	0.153	0.146	.....	.....	.....	0.125	0.124	0.125
Lactose	1	.....	.....	3.65*	3.70*	.....	.....	.....	5.30	5.05	5.25
	2	.....	.....	3.75*	4.15	.....	.....	.....	4.80*	4.45*	2.30*
	3	.....	.....	4.40	4.20	.....	.....	.....	5.30	5.00	5.35
	4	.....	.....	4.65	4.00	.....	.....	.....	5.05	4.90	5.00
Chlorine-lactose number	1	.....	.....	4.68*	4.70*	.....	.....	.....	2.17	2.36	2.13
	2	.....	.....	4.29*	3.31	.....	.....	.....	2.81*	3.48*	10.95*
	3	.....	.....	3.16	3.05	.....	.....	.....	2.09	2.36	1.91
	4	.....	.....	3.29	3.65	.....	.....	.....	2.47	2.53	2.50
Casein number	1	77.9	76.0	76.6	74.8*	.....	.....	.....	77.9	78.8	77.8
	2	77.3	75.0	77.5	78.2	.....	.....	.....	75.7	75.8*	66.7*
	3	78.7	78.0	79.1	78.4	.....	.....	.....	77.9	79.7	78.5
	4	78.6	76.4	77.7	76.7	.....	.....	.....	76.5	78.8	77.4

TABLE 4.—(*Continued*)

Cow #360, Fourth and Fifth Lactations

	Number of quarters	Months of lactation									
		9	10	Dry	Dry	1	2	3	4	5	6
Diagnosis	1	–	S	.....	.....	–	–	–	–	–	–
	2	–	S	.....	.....	S	S	S	S	+	+
	3	–	S	.....	.....	–	S	–	–	–	–
	4	–	S	.....	.....	–	S	+	+	+	S
Chlorine	1	0.150	0.166	.....	.....	0.106	0.118	0.128	0.119	0.120	0.123
	2	0.184*	0.218*	.....	.....	0.158*	0.131	0.128	0.157*	0.173*	0.184*
	3	0.143	0.173	.....	.....	0.108	0.113	0.126	0.119	0.127	0.120
	4	0.161	0.186*	.....	.....	0.112	0.131	0.147*	0.158*	0.139	0.212*
Lactose	1	.....	.....	.....	.....	5.35	5.30	5.07	5.10	5.10	5.05
	2	.....	.....	.....	.....	4.55*	5.10	4.73	4.45*	4.20*	4.00*
	3	.....	.....	.....	.....	5.30	5.40	5.05	5.20	5.05	5.10
	4	.....	.....	.....	.....	5.25	5.10	4.65*	4.55*	4.80	3.55*
Chlorine-lactose number	1	.....	.....	.....	.....	1.98	2.23	2.52	2.33	2.35	2.44
	2	.....	.....	.....	.....	3.47*	2.57	2.71	3.53*	4.12*	4.60*
	3	.....	.....	.....	.....	2.04	2.09	2.50	2.28	2.51	2.35
	4	.....	.....	.....	.....	2.13	2.57	3.16	3.47*	2.89	5.97*
Casein number	1	77.3	72.9	.....	.....	78.9	78.3	76.7	76.1	77.6	75.6
	2	74.3*	63.6*	.....	.....	74.5*	75.4*	73.3*	70.8*	69.3*	66.4*
	3	78.4	71.3	.....	.....	78.9	77.9	76.9	76.0	76.8	73.9
	4	76.0	68.7*	.....	.....	78.6	76.7	74.4	73.7	75.0	63.0*

* Milk abnormal composition.
\+ Positive for mastitis.
– Negative for mastitis.
S Suspicious for mastitis.

was diagnosed as suspicious. During the twelfth month the second quarter was diagnosed as positive and the others as suspicious. Quarter number 1 secreted abnormal milk high in chlorine and low in lactose during the eleventh and twelfth months of the fourth lactation. The second quarter secreted abnormal milk during the eleventh but apparently not during the last month of the lactation period. During the first months of the fifth lactation, the second quarter was diagnosed as positive while the other quarters were considered as suspicious. The first quarter did not continue to secrete abnormal milk after the intervening dry period between the fourth and fifth lactations which indicated improvement in the condition of the quarter during the dry period. However, the second quarter which was diagnosed as positive secreted abnormal milk high in chlorine and low in lactose from the first to the third month. The condition also became increasingly worse from the first to the third month.

Cow number 360 secreted milk of abnormal chemical composition from time to time in the second and fourth quarters during the latter part of the fourth lactation, and the first six months of the fifth. In the case of this animal there is some evidence that quarters may show some improvement and at times secrete milk of normal composition, while at other times the milk may be definitely abnormal. The second and fourth quarters were diagnosed as either suspicious or positive of mastitis.

DISCUSSION

From an examination of the data considerable evidence was obtained which indicates that certain micro-organisms generally considered to be the causal agents responsible for mastitis may be shed in the milk without any change in the chlorine, lactose or casein number of the milk at that particular time. The length of time which may elapse after the invasion of these organisms before any significant change in the chlorine, lactose and casein number is apparent, varies with different animals and with different quarters of the same animal. Many instances were observed where certain quarters were diagnosed positive during the latter part of the period but did not show a significant change in the chlorine, lactose or casein number during that particular lactation. This condition did not eliminate the possibility that eventually changes in these constituents of the milk might become apparent during the next lactation. However, certain individuals shedding these organisms during the latter part of lactation began the next lactation without any indications of mastitis.

It is evident that the biochemical tests used will not indicate quarters showing incipient stages of chronic mastitis.

Significant changes in the chlorine, lactose or casein number of the foremilk based upon quarter differences were almost universally associated with mastitis, but on the other hand many quarters were diagnosed as positive

for mastitis which did not show significant difference between diseased and normal quarters at that particular time. Apparently there must be a definite change of the mammary tissue before the composition of the milk of the individual quarter is affected. Further evidence for this statement might be obtained by histological examinations of udders which are known to have secreted milk of abnormal composition.

The data also indicate that changes in the percentage of chlorine, lactose, chlorine-lactose number and casein number take place simultaneously. There were a few exceptions, but the possibility of analytical error was not entirely eliminated. Since some of these constituents may be derived directly from the blood by diffusion through the cell membranes, while others must be synthesized in the mammary gland, it appears as though in mastitis there must be a change in the synthetic and secretory mechanism of milk secretion as well as an increase in the permeability of the cell membranes.

For detecting chemical changes in the milk due to or associated with mastitis, the determination of the casein number was no more desirable than a determination of either the chlorine or the lactose. The determination of the chlorine and lactose with the calculation of the chlorine-lactose number is to be preferred over either chlorine or lactose alone.

Changes in the percentage of chlorine and lactose with corresponding changes in the chlorine-lactose number based upon quarter differences in the same udder can be recommended as an indirect biochemical method for detecting chronic mastitis which has developed to the stage of causing changes in the composition of the milk of affected quarters.

Diagnosis of chronic mastitis by changes in the percentage chlorine, lactose, chlorine-lactose number or casein number due to or associated with mastitis and based upon quarter differences instead of a set standard, practically eliminates difficulty due to changes in the chemical composition of the milk from time to time as well as changes associated with age and advanced stages of lactation and certain peculiarities of the individual animals.

SUMMARY AND CONCLUSIONS

In this study approximately 250 udder examinations on 40 purebred Holstein cows were made. The percentages of chlorine, lactose, total nitrogen and non-casein nitrogen were determined on samples of fore-milk from individual quarters, and from these the chlorine-lactose number and the casein number were calculated.

Physical examination and the following diagnostic tests on samples of fore-milk were made in conjunction with the chemical studies: strip cup, brom thymol blue, Hotis test, chlorine (colorimetric), microscopic examination of incubated milk, leucocyte count, and blood agar plate.

The fore-milk from individual quarters free from mastitis was found to

contain from 0.090 to 0.224 with an average of 0.124 per cent chlorine, and from 3.30 to 5.40 with an average of 4.788 per cent for lactose. The chlorine-lactose number varied from 1.62 to 6.79 with an average of 2.614, while the casein number varied from 69.4 to 84.3 with an average of 77.57.

Milk secreted by quarters in the advanced stages of mastitis contained a higher percentage of chlorine and a lower percentage of lactose than milk from normal quarters. The chlorine-lactose number was also higher and the casein number lower in milk from quarters with mastitis than from normal quarters.

The detection of changes in the chlorine, lactose or casein number of milk due to or associated with mastitis based upon quarter differences practically eliminates difficulty due to changes in the chemical composition of the milk from time to time as well as changes associated with age, advanced stages of lactation, and certain peculiarities of the individual animals.

The mean difference between quarters in normal udders free from mastitis was for chlorine content 0.0065 per cent, lactose 0.115 per cent, chlorine-lactose number 0.184, and casein number 0.999.

In order to detect affected quarters by changes in the chemical composition of the milk from individual quarters, the quarter with the lowest per cent chlorine and chlorine-lactose number, or the highest per cent lactose and casein number was considered normal. For a significant difference between normal quarters and affected quarters there must be a difference of at least 0.02 per cent chlorine, 0.36 per cent lactose, 0.60 for chlorine-lactose number, and 2.90 for casein number when samples of six to eight ounces of milk were used.

Individual quarters usually showed bacteriological changes in the foremilk before a change in the chemical composition of the milk was apparent.

Indirect biochemical tests such as the percentage of chlorine, the percentage of lactose, the chlorine-lactose number or the casein number when based upon quarter differences in the same udder can be recommended for the diagnosis of chronic mastitis. However, these tests will not indicate quarters which show chronic mastitis in the incipient stages.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Dr. E. J. Wellhausen, of the Department of Agronomy and Genetics, for many helpful suggestions in the statistical treatment of the data.

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THE EFFECT OF COCOA UPON THE DIGESTIBILITY OF MILK PROTEINS

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INTRODUCTION

Before any substance is added to milk, one should be certain that it will not destroy some of the well-known nutritive properties of plain milk. No doubt the increase in milk consumption, especially by children, as a result of adding chocolate flavoring would be desirable, provided it did not injure the nutritional properties of the milk.

Mueller and Ritchie (1) found that the addition of 4 per cent by weight of cocoa to plain milk, lowered the gain in weight of rats by approximately 17 per cent, even though the amount of milk consumed was the same as when a plain whole milk diet was fed. It was thought that this observed decrease in rate of growth might be due in part to a decrease in the digestibility of the milk proteins as a result of adding cocoa.

Ulrich (2) found that cacao beans may contain approximately 8 per cent by weight of cacao red. In its reactions with proteins, cacao red much resembles tannin. Whymper (3) states that tannin decreases the solubility of milk solids.

Neumann (4, 5) studied the digestibility of cocoa, using himself as a subject for 86 days, and reported that the addition of cocoa to other articles of food seemed to reduce the total amount of nitrogen absorbed. He also found that the amount of fat present in cocoa affected the absorption of nitrogen, a reduction in fat lowering the assimilation of nitrogen.

Aplin and Ellenberger (6), working with cows, found that the addition of two pounds of cocoa meal (a by-product of the chocolate industry) to the daily amount of food consumed by the cows reduced the coefficient of digestibility of the crude protein from 55 per cent for the cocoa-free food to 50 per cent for the diet containing cocoa.

Mitchell *et al.* (7), studying the digestibility of cocoa and milk proteins, concluded that no marked supplementary relation exists between the nitrogenous compounds of milk and of cocoa. In their studies 50 per cent of the nitrogen in the food used came from the cocoa and the other 50 per cent from the milk.

Received for publication December 30, 1940.

[1] This article is based upon a Master's thesis presented to the Graduate School by L. D. Lipman, June, 1939.

Published as Contribution No. 365 of the Massachusetts Agricultural Experiment Station.

OBJECT OF STUDY

This study was undertaken with the hope of demonstrating by animal feeding experiments whether or not the addition of cocoa to whole milk powder (in amounts equivalent to approximately 4 per cent by weight on a fluid milk basis) had any adverse effect upon the digestibility of the milk proteins. The effect of both the Dutch-process and the American-process cocoa as well as the effect of added cocoa fat was studied.

It was also hoped that the data obtained in this study would aid in explaining the cause for the retarded rate of growth of white rats, as reported by Mueller and Ritchie (1).

EXPERIMENTAL

Determining Digestibility of Proteins in Milk and Milk Plus Cocoa. In order to study the effect of cocoa upon the digestibility of the milk proteins, the following determinations had to be made: 1, Digestibility of proteins in milk alone; 2, Digestibility of proteins in milk plus cocoa; 3, Digestibility of the cocoa proteins in the absence of milk proteins; and 4, Metabolic nitrogen. Twenty-four white rats from the same colony and nine weeks old were used for the entire study.

In the first study the rats were divided into four groups, each group consisting of six rats, three males and three females, except group IV, which consisted of two females and four males. The groups were arranged so that the average weight was approximately the same.

The feed formulas for the four groups are given in table 1, which also gives the total nitrogen as determined by the Kjeldahl method. It should be noted that these diets are equivalent on a fluid milk basis to approximately 3.6 per cent cocoa, 6.3 per cent cane sugar and 13 per cent milk solids. Whole milk powder was used in this study instead of fluid milk, so that all of the feed for a trial could be prepared at the same time. This insured that the composition of the feed would not vary from day to day as might be the case if fresh fluid milk were used. Also, milk powder from the same batch could be used for a number of trials, thus insuring that the milk proteins used in all trials had received the same previous treatment. Another advantage of the dry feed is that the cocoa powder will stay evenly distributed and will not settle as it does when added to fluid milk. A third advantage is that the uneaten food could be determined by weight, whereas the amount of fluid milk left cannot be determined so accurately, due to losses by evaporation.

Group I is the control group, which was fed the basal diet consisting of whole milk powder, sugar, and salt mixture (Osborne and Mendel). Group II was fed a diet containing the same constituents as Group I, but containing 15.8 per cent Dutch-process cocoa. The diet for Group III was the same as for Group II except that American-process cocoa was used instead of the Dutch-process cocoa. Group IV's diet contained the same constituents as

TABLE 1

Feed formula used in determining digestibility of milk and milk plus cocoa proteins

Ingredient	Group I	Group II	Group III	Group IV
	%	%	%	%
Whole milk powder	62.9	52.6	52.6	48.7
Cane sugar	33.1	27.6	27.6	25.6
Cocoa	.....	15.8 (D)	15.8 (A)	14.6 (A)
Cocoa fat	.....	.....	.....	7.1
Osborne and Mendel salt mixture	4.0	4.0	4.0	4.0
Cocoa on fluid milk basis	0.0	3.6	3.6	3.5
Total nitrogen	2.45	2.49	2.50	2.48

(A) Indicates American-process.
(D) Indicates Dutch-process.

Group III's, except that pure cocoa fat was added, in order to raise the cocoa fat content to the same level as if chocolate liquor had been used.

The rats were individually caged and were fed the diet ad libitum for a period of 18 days to thoroughly accustom them to it. During this period, various preliminary tests were carried out. The feed was placed in Fisher porcelain cups of 50 cc. capacity which were in turn placed in large metal feed cups with covers. The purpose of the metal feed cup was to catch the feed which was spilled from the porcelain cups by the animals. The rats had water before them at all times.

The rats were fed 10 grams of feed daily during the test period of three days, as preliminary trials showed that they consumed about 10 grams of feed daily. The first feeding of 10 grams was marked with carmine. Paper towels were placed under the grid floor of the cages to catch the feces, and the feces marked with carmine (red) were collected. Ten more grams of feed were fed on both the second and third days. On the fourth day, the amount of feed left, including that spilled into the metal feed cups was weighed and the total amount of feed consumed was determined. The cups were cleaned, then feed marked with charcoal was given the rats and the feces were collected until the marked feces came through (black). Each rat's feces were collected daily and stored separately in a refrigerator (0° F.). The feces from each rat were weighed and ground in a mortar. A 2-gram sample of the ground feces was analyzed for total nitrogen by the Kjeldahl method, and the total amount of nitrogen in the feces calculated. The animals were weighed on the first and fourth days of the test period. The results of this experiment are shown in table 4.

Determining Digestibility of Proteins in Cocoa. In this experiment the feeds for Groups II, III, and IV were made up to contain approximately the same amounts of cocoa and cocoa fat on a percentage basis as feeds described in the preceding experiment. The same rats were used as before and were kept in the same groups. The only source of protein in the diet was the cocoa, while in the previous experiment the protein was furnished by both

cocoa and milk powder except in Group I, which contained only milk proteins. The composition of the feeds is given in table 2, which also gives the amount of nitrogen. The rats were fed ad libitum for three days previous to the test. During the test period of three days the rats were fed 10 grams of feed daily. The food was marked, the feces collected and stored, and the total nitrogen consumed and excreted was determined as before. The animals were weighed on the first and fourth days of the test period. Results of this study are shown in table 5.

TABLE 2

Feed formula used in determining digestibility of cocoa proteins

Ingredient	Group II	Group III	Group IV
	%	%	%
Corn starch	68.5	68.5	62.4
Osborne and Mendel salt mixture	4.0	4.0	4.0
Butter fat	9.0	9.0	9.0
Cod liver oil	2.0	2.0	2.0
Cocoa	16.5 (D)	16.5 (A)	15.2 (A)
Cocoa fat	.....	.....	7.4
Total nitrogen	0.74	0.74	0.60

(D) Indicates Dutch-process.
(A) Indicates American-process.

Determining the Metabolic Nitrogen. The fecal metabolic nitrogen is the small amount of nitrogen that is found in the feces when the animal is fed a low-protein diet. It is composed of epithelial cells, bacteria, mucus and the residues of the bile and digestive juices. Theoretically, during the determination of the fecal metabolic nitrogen, the rats should be fed on a non-protein diet. However, the work of various investigators has shown that the rats must be fed at least 4 per cent of protein or they will lose weight very rapidly and die within a short time. In his earlier work Mitchell (8) fed a protein-free diet while determining metabolic nitrogen, but in later experiments (9, 10), a diet containing small amounts of protein was used. Kon (11) and Schneider (12) also used a low-protein diet in determining metabolic nitrogen.

TABLE 3

Feed formula used in determining metabolic nitrogen

Ingredient	Groups I, II, III	Group IV
	%	%
Corn starch	80.0	74.5
Osborne and Mendel salt mixture	4.0	3.7
Butter fat	9.0	8.4
Cod liver oil	2.0	1.9
Casein	5.0	4.6
Cocoa fat	.....	6.9
Total nitrogen	0.91	0.79

The composition and total nitrogen of the diets used for determining metabolic nitrogen are given in table 3. The rats were fed the food for three days before the test period. During the test period which lasted three days, the rats were fed 10 grams of feed daily. The food was marked, amount consumed determined, feces were collected, and the total nitrogen excreted was determined as before. All animals were weighed on the first and fourth days of the test period. The results are shown in table 6.

In determining the value for the fecal metabolic nitrogen, to be used in

TABLE 4

Digestibility of the proteins in the various diets

Rat no.	Initial weight	Final weight	Food intake	Nitrogen intake	Nitrogen in feces	Metabolic nitrogen in feces	Food nitrogen in feces	Nitrogen digested
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
Group I—62.9 per cent milk powder								
1	140	141	23.5	.576	.135	.046	.089	84.5
2	150	146	30.0	.737	.170	.053	.115	84.4
3	148	153	28.0	.687	.180	.054	.126	81.7
4	171	171	29.8	.731	.167	.068	.099	86.5
5	173	174	28.2	.692	.144	.055	.089	87.1
6	180	178	29.9	.733	.149	.059	.090	87.7
								Av. 85.3
Group II—52.6 per cent milk powder and 15.8 per cent Dutch-process cocoa								
7	130	128	29.6	.737	.303	.057	.246	66.7
8	131	130	29.7	.740	.251	.063	.194	73.6
9	138	138	30.0	.747	.301	.060	.241	67.9
10	146	154	30.0	.747	.238	.054	.184	76.7
11	152	157	30.0	.747	.335	.056	.279	62.7
12	157	155	29.9	.745	.316	.059	.257	65.5
								Av. 68.9
Group III—52.6 per cent milk powder and 15.8 per cent American-process cocoa								
13	144	140	28.3	.706	.283	.054	.229	67.7
14	155	152	29.8	.744	.238	.057	.181	75.7
15	170	166	29.9	.746	.215	.059	.156	79.1
16	161	160	30.0	.749	.332	.060	.272	63.6
17	184	180	30.0	.749	.259	.058	.201	73.0
18	192	175	30.0	.749	.302	.060	.242	67.8
								Av. 71.2
Group IV—48.7 per cent milk powder and 14.6 per cent American-process cocoa and 7.1 per cent cocoa fat								
19	116	116	30.0	.745	.221	.059	.162	78.3
20	166	163	29.9	.742	.272	.062	.210	70.4
21	158	156	30.0	.745	.264	.059	.205	72.6
22	154	147	30.0	.745	.280	.063	.217	70.9
23	160	165	29.9	.742	.288	.061	.227	66.8
24	171	167	30.0	.745	.300	.059	.241	67.7
								Av. 71.1

calculating the digestibility of the food proteins, the metabolic nitrogen was corrected by multiplying the metabolic nitrogen per gram of low-protein food by the number of grams of food consumed during the trial under consideration. This correction is necessary since Mitchell (10), and later Schneider (12) have shown that the metabolic nitrogen is proportional to the amount of food consumed.

TABLE 5

Digestibility of proteins in cocoa

Rat no.	Initial weight	Final weight	Food intake	Nitrogen intake	Nitrogen in feces	Metabolic nitrogen in feces	Food nitrogen in feces	Nitrogen digested
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
Group II—16.5 per cent Dutch-process cocoa								
7	120	113	26.2	.193	.156	.050	.106	45.1
8	131	118	29.7	.220	.206	.063	.143	35.0
9	125	120	29.6	.219	.177	.060	.117	46.9
10	144	137	22.6	.167	.154	.042	.112	32.9
11	150	138	28.1	.207	.195	.053	.142	31.3
12	144	140	28.0	.207	.185	.055	.130	37.2
								Av. 38.1
Group III—16.5 per cent American-process cocoa								
13	147	128	28.0	.207	.185	.054	.131	37.0
14	143	133	28.5	.211	.176	.053	.123	41.5
15	161	149	29.1	.215	.150	.059	.109	58.1
16	148	142	26.1	.193	.159	.052	.107	44.9
17	175	165	29.5	.218	.179	.058	.121	44.7
18	180	163	26.9	.199	.172	.054	.118	41.0
								Av. 44.5
Group IV—15.2 per cent American-process cocoa and 7.4 per cent cocoa fat								
19	114	107	23.5	.141	.128	.047	.081	42.6
20	155	146	29.5	.177	.152	.062	.090	49.0
21	153	140	27.3	.163	.169	.053	.116	29.2
22	141	135	24.7	.148	.134	.052	.082	45.0
23	153	143	28.7	.172	.170	.059	.111	35.8
24	160	148	24.9	.149	.131	.049	.082	44.8
								Av. 41.1

DISCUSSION OF RESULTS

The effect of cocoa on the digestibility of the milk proteins is shown in table 7. The following formula was used in making the calculations:

$$D = \frac{(C-B)(X)+B(A)}{C}$$

Solving for X:

$$X = \frac{C(D)-B(A)}{(C-B)}$$

Where X = digestibility of milk proteins
D = digestibility of mixture of cocoa proteins and milk proteins
A = digestibility of cocoa proteins
B = total nitrogen in feed from cocoa
C = total nitrogen in feed
C − B = total nitrogen in feed from milk

The analysis of the American- and Dutch-process cocoas for total nitrogen gave values of 0.0382 and 0.0367 grams per gram of cocoa powder respectively.

A value of 85.3 per cent was obtained for the digestibility of milk proteins in whole milk powder. This value is somewhat lower than the average which has been reported in the literature by various investigators. Fairbanks and Mitchell (13), and Nevens and Shaw (14) have shown that the

TABLE 6

Determination of metabolic nitrogen

Rat no.	Initial weight	Final weight	Food intake	Nitrogen in feces (metabolic)
	grams	*grams*	*grams*	*grams*
		Group I		
1	137	135	28.2	.053
2	148	146	29.8	.055
3	152	148	29.0	.054
4	171	166	25.0	.057
5	171	166	23.4	.047
6	182	178	28.2	.059
		Group II		
7	119	119	30.0	.059
8	119	116	25.0	.053
9	113	112	30.0	.060
10	136	131	14.0	.025
11	144	141	21.0	.039
12	142	135	24.0	.047
		Group III		
13	134	129	23.0	.044
14	145	139	28.0	.053
15	154	145	20.0	.040
16	139	134	30.0	.060
17	163	159	25.0	.048
18	164	158	30.0	.060
		Group IV		
19	103	102	23.0	.045
20	150	148	30.0	.062
21	146	145	27.0	.053
22	135	134	30.0	.063
23	142	147	29.0	.059
24	159	141	23.0	.045

digestibility of the milk proteins is lowered by the heat treatment to which the milk is subjected during the drying process. Therefore, considerable variation in the digestibility of proteins in various milk powders may be expected. Mitchell (8) found that there is a slight decrease in the digestibility of milk proteins as the protein level is increased from 5 to 10 per cent. In the present study the protein level fed was 15 per cent, which may be a contributing factor for the somewhat low value of 85.3 per cent.

TABLE 7

Effect of cocoa on the digestibility of milk proteins

Kind of diet	Average digestibility of milk proteins	Difference when compared with plain milk	Decrease when compared with plain milk
	per cent probable error		*per cent*
Basal ration (whole milk powder, cane sugar and salt mixture)	85.3 ± 0.56	.....	.....
Basal ration including 15.8 per cent Dutch-process cocoa	78.6 ± 1.90	− 6.7	7.85
Basal ration including 15.8 per cent American-process cocoa	80.1 ± 1.63	− 5.2	6.09
Basal ration including 14.6 per cent American-process cocoa and 7.1 per cent cocoa fat	80.3 ± 1.46	− 5.0	5.86

The calculated digestibility (see table 7) of the milk proteins in diets containing approximately 15.8 per cent of cocoa, show that the Dutch-process cocoa lowered the percentage digestibility from 85.3 (± .56) to 78.6 (± 1.90), while the American-process cocoa lowered the percentage digestibility from 85.3 (± .56) to 80.1 (± 1.63). Thus no significant difference was noted for the two kinds of cocoa. Also the inclusion of 7.1 per cent by weight of cocoa fat to the milk powder–American-process cocoa ration, did not alter to any marked extent the digestibility of the milk proteins. When the same method of calculation as used in this study is applied to the results obtained by Mitchell (7) the values obtained show that the percentage digestibility of the milk proteins is lowered from 95 to 88 when Dutch-process cocoa was added to the diet.

The results in this study that cocoa decreases the digestibility of the milk proteins is in agreement with Neumann's (4, 5) report that the addition of cocoa to other articles of food seemed to reduce the total amount of nitrogen absorbed. However, it should be mentioned that Neumann's experiments were performed on a human subject, whereas white rats were used in this study.

Although the observed decrease in digestibility of milk proteins when cocoa was added is not marked, no doubt it is of some significance in explaining the results by Mueller and Ritchie (1). These investigators found that

the addition to a whole milk powder ration of 17.8 per cent by weight of Dutch-process cocoa, equivalent to 4 per cent by weight on fluid milk basis, lowered the rate of weight gain in rats by 17 per cent, even though the amount of milk powder consumed was the same as when a plain whole milk powder diet was fed.

It will be noted in table 7 that the probable error for the digestibility of milk proteins is greater when cocoa is added to whole milk powder as compared to whole milk powder alone. This is to be expected since the determination of the digestibility of the milk proteins in the presence of cocoa is a more complicated procedure than when milk powder is the only source for the proteins. Furthermore, it is readily conceivable that the decrease in palatability of the ration, as a result of including 15.8 per cent by weight of cocoa may be a contributing factor in increasing the probable error.

The digestibility of the proteins in cocoa is of little practical significance in chocolate milk, due to the small amount of cocoa used. However, it was necessary to determine the digestibility of the cocoa proteins in this study, in order to calculate the digestibility of the milk proteins when cocoa was added to the milk powder. The value obtained for the digestibility of the protein from Dutch-process cocoa was 38.1 per cent, and agrees with that obtained by Mitchell *et al.* (7). A value of 44.5 per cent was obtained for the digestibility of the protein in American-process cocoa, and this value was lowered to 41.1 per cent when 7.4 per cent by weight of cocoa fat was included in the ration.

SUMMARY AND CONCLUSIONS

Whole milk powder plus a commercial brand of Dutch-process cocoa and whole milk powder plus a commercial brand of American-process cocoa, with and without additional cocoa fat, were fed in comparison with whole milk powder in feeding trials with albino rats. The digestibility of the milk proteins was studied.

The rats were able to digest approximately 85, 69, 71, and 71 per cent of the food proteins, when rations containing 62.9 per cent milk powder, 52.6 per cent milk powder and 15.8 per cent Dutch-process cocoa, 52.6 per cent milk powder and 15.8 per cent American-process cocoa, 48.7 per cent milk powder and 14.6 per cent American-process cocoa and 7.1 per cent cocoa fat were fed, respectively. Subjecting these results to mathematical analysis revealed that the digestibility of milk proteins (85.3 per cent) was reduced 7.8 and 6.0 per cent when the ration contained 15.8 per cent Dutch, and 15.8 per cent American-process cocoa, respectively. The inclusion of 7.1 per cent of cocoa fat to the American-process cocoa–milk rations reduced the digestibility of the milk proteins by 5.8 per cent.

The proteins of the American-process cocoa were more completely digested (44.5 per cent) than those of Dutch-process (38.1 per cent), when the

ration contained 16.5 per cent of cocoa and cocoa was the only source of protein in the diet. The digestibility of the proteins in the American-process cocoa was found to be only 41.1 per cent when 7.4 per cent by weight of cocoa fat was included in the ration.

On the basis that the addition of cocoa to whole milk powder (in quantity equivalent to approximately 3.6 per cent by weight on a fluid milk basis) does not greatly reduce the digestibility of the milk proteins, we may conclude that the amount of cocoa in average commercial chocolate milk (approximately 1 per cent by weight) has no significant adverse effect upon the digestibility of the milk proteins.

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CARE OF MILKING MACHINES[1]

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In a recent paper by Mallman, Bryan and Begeman (3) it is stated: "There is a dearth of information on methods of cleaning milking machines. Most publications place emphasis on the sanitation. The most common practice in cleaning consists of flushing machines with cold water after each milking and then placing the tubes on the racks for either alkali or chlorine treatment. Under these conditions machines are washed once a week with hot water and a detergent. This procedure is very poor operating practice." Several of these statements should not be allowed to pass unquestioned. That "there is a dearth of information on methods of cleaning milking machines" is difficult to reconcile with the extensive literature on the subject. The assertion that flushing tubes with cold water, then filling with alkali solution "is very poor operating practice" should be supported by definite evidence since it is at variance with the results reported by several investigators (1, 2, 4, 5). Finally, the inference that tubes are not considered properly washed unless hot detergent solution is used after each milking also appears to run counter to the facts.

Until 1932 the need for a suction rinse with hot detergent solution was generally admitted. However, as the above-mentioned authors admit, this "is seldom carried out in practice, and even where it is used the method is not carried out with sufficient thoroughness to render the equipment free from milk wastes and lime deposits." In 1932, however, it was shown (2) that a cold lye solution, acting during the long interval between milkings, maintains the rubber tubing, etc., in as clean condition as does a brief rinse with hot detergent solution. The lye solution should therefore be regarded as a detergent as well as a germicide. On the other hand, where the tubes are rinsed with cold water, then filled with chlorine solution (which has no appreciable detergent action) the results are much less satisfactory (2). Presumably the objectionable conditions referred to by Mallmann *et al.* were due to the use of chlorine in place of lye, although their blanket condemnation of the cold water rinse method applies to both lye and chlorine solutions.

The efficiency of the lye solution method, following a cold suction rinse, in maintaining milker tubes in satisfactory bacteriological condition has been demonstrated by Johns (2), by Hastings and Werner (1), and by Parfitt (4). Rogers and Evans (5) have likewise shown that immersion between

Received for publication January 6, 1941.

[1] Contribution No. 120 (Journal Series) from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa.

milkings in a cold solution of trisodium phosphate maintains the tubes in a satisfactory state. Further evidence of the effectiveness of the lye solution method is presented in table 1, based upon routine counts obtained on the milk from the Central Experimental Farm Dairy between January 1, 1938, and December 31, 1939. (Filling the tubes with weak lye solution following a suction rinse with cold water, has been standard practice since August, 1930). The counts here summarized represent routine plate counts on samples of mixed night's and morning's milk taken from the pasteurizing vat before heating, hence contamination from various sources other than the milking machines is included. It will be observed that 80.7 per cent of the counts were below 5,000 per ml. and 94.2 per cent below 10,000 per ml. The highest count recorded was 29,000.

TABLE 1

Distribution of plate counts on routine raw milk samples (C. E. F. dairy, 1938–1939)

	Bacteria counts*							Total
	< 2,500	2,501–5,000	5,001–7,500	7,501–10,000	10,001–15,000	15,001–25,000	25,001–50,000	
Number	81	87	18	10	6	5	1	208
Percentage	38.9	41.8	8.7	4.8	2.9	2.4	0.5	100

* Standard methods of A.P.H.A. followed, except that tryptone-glucose-skim milk agar was introduced January 1, 1939, instead of July 1.

TABLE 2

Monthly average (logarithmic) counts on routine samples of raw and pasteurized milk (C. E. F. dairy)

Month	No. of samples	Raw milk			Pasteurized milk
		Plate count	Coliform bacteria* in		Plate count
			0.1 ml.	0.01 ml.	
1933			%	%	
September	7	5,430	71.5	35.7	97
October	9	19,240	37.7	17.0	142
November†	7	10,530	57.1	28.6	147
December	5	6,850	70.0	20.0	63
1934					
January	8	8,350	12.5	0.0	58
February	7	6,950	0.0	0.0	30
March	8	5,370	28.5	0.0	38
April	7	17,200	14.3	0.0	33
May†	9	6,220	66.7	22.2	23
June	10	10,330	75.6	40.0	36
July	8	6,960	81.0	31.0	17
August	5	13,440	100.0	100.0	16

* Percentage of tubes of brilliant green lactose bile broth showing gas.

† Between Nov. 27 and May 4, two units received no rinse of any sort, going direct from cow to solution rack where they were filled with 0.5% lye solution.

Still more striking evidence of the detergent action of weak lye solution was obtained some years ago. Due to a misunderstanding, two milker units failed to receive even a cold water rinse at the barn, being placed directly on the solution rack and filled with 0.5 per cent lye solution. The usual weekly disassembling and brushing with hot detergent was also omitted. This continued from November 27, 1933, to May 4, 1934, at which time it was necessary to replace the liners. Data from routine analyses of both raw and pasteurized milk during the period September, 1933, to August, 1934, are summarized in table 2. At the conclusion of these studies, some calcium phosphate from the milk residue was found deposited in the liners and tubes. This condition was not reflected in higher bacteria counts in either raw or pasteurized milks, despite the claim (3) that no chemical disinfection will function when the surface to be disinfected is covered with such deposits. The omission of all rinsing to remove the residual milk is definitely not to be recommended, but the results show very clearly how effective the lye solution is, both as a detergent and as a germicide. This is confirmed by the results from a number of farms where this method is employed.

Since all available evidence indicates that milker tubes can be kept in good sanitary condition by filling with lye solution following a cold water rinse, it seems difficult to justify the recommendation that machines be given an additional rinse with hot detergent solution. While the additional rinse will admittedly lower the bacteria count slightly (2, 4) this extra step would only be justified where measures were being taken to reduce bacterial contamination to the absolute minimum. The level of bacteria counts reported by Mallmann *et al.* (3) (geometrical mean of 172,000 per ml.) for milk drawn by machines subjected to a "satisfactory" method of cleaning suggests that the milk supplies which they investigated were scarcely in this category.

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THE pH VALUES OF THE INGESTA OF THE RUMEN OF SLAUGHTERED ANIMALS[1]

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In our work on bloat in bovines, we visited several packing plants to obtain samples of rumen gas. After the gas had been obtained the rumen was slit open, and a four-ounce sample of the rumen contents was taken. Samples of ingesta were also obtained from animals when no gas samples had previously been taken. No effort was made to obtain the ingesta in any particular part of the rumen. The sample consisted largely of the juice. When the juice was difficult to obtain by dipping, a quantity of ingesta was squeezed in the hand.

The juice was taken to the laboratory, where the pH was determined on a Coleman's electric potentiometer. In several instances the pH was determined the afternoon of the same day on which the ingesta samples were collected. In other cases the pH values were not determined until approximately 20 hours after the samples were taken.

The animals from which the samples were taken had been purchased for slaughter. Many of them were yard fed, while others were grass fed before being received at the plant. As is customary when animals are delivered to the yards for slaughter, they are fed a limited quantity of roughage, usually a non-legume such as prairie hay.

Whenever it was possible to identify the type of feed or grain in the ingesta, this was done. Obviously it was not possible to identify all the grains, or any finely ground feed.

TABLE 1

	No. of samples	Time elapsed before pH was determined	Range	Average
		hr.	*pH*	*pH*
Dec. 1, 1939	104	22	7.59–6.41	6.87
" 27, 1939	55	22	7.40–6.41	6.98
" 29, 1939	51	5	7.20–5.70	6.65
" 30, 1939	48	5	7.13–6.16	6.76
Jan. 3, 1940	37	22	7.45–5.90	6.99
" 4, 1940	55	7	7.78–5.52	6.89
June 6, 1940	8	8	7.56–6.89	7.07
" 6, 1940	42	8	7.39–5.97	6.81
" 18, 1940	41	7	7.45–6.08	6.88
July 3, 1940	32	45	7.55–5.56	6.89
	473			Av. 6.859

Received for publication January 10, 1941.

[1] South Dakota Agricultural Experiment Station, Journal Series No. 145.

TABLE 2

Average pH values of ingesta held under room temperatures for a given period following collection

Sample no.	Number of samples	5 hr.	7 hr.	8 hr.	22 hr.	30 hr.	45 hr.	70 hr.	95 hr.	120 hr.	150 hr.	168 hr.	235 hr.
		pH	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*	*pH*
1	104				6.87							7.57	
2	55				6.98					7.30			
3	51	6.65										7.07	
4	48	6.76										6.95	
5	37				6.99							8.29	
6	55		6.89			6.97		7.22	7.49				
7	8			7.07	7.25		7.31		7.77		8.07		
8	42			6.81	7.04		7.13		7.64		7.92		
9	41		6.88		6.86		7.00		7.47		7.45	7.51	
10	32						6.89			7.22		7.70	7.96
	473	6.70	6.88	6.85	6.95	6.97	7.02	7.22	7.54	7.27	7.72	7.49	7.96

RESULTS

Samples of ingesta were collected from slaughtered animals on the following dates, and the pH values determined at the stipulated time. (See table 1.)

The samples of ingesta were held at room temperature for various lengths of time and the pH values determined to note what changes if any in the pH followed the respective holding periods. The data in table 2 indicate the results.

DISCUSSION

The samples of ingesta were obtained from cows, steers and a few from aged bulls. Some of these were of beef strains, others of dairy. Some were choice beef animals, others were canners. No significant differences in pH values were obtained in these various animals.

The animals on which notations on the feed in the rumen at time of slaughter were made, did not reveal data from which definite conclusions on the effect of feed on pH can be drawn. It did seem, however, that those animals which showed hay or straw only in the rumen had a somewhat higher pH value. Yet in many instances the pH values were no higher than when grain was present with the roughage. In sample No. 7 eight samples of ingesta were obtained from cows whose ingesta indicated they had been fed on green grass. These were more alkaline than the average of the other samples.

The pH values on the 473 samples averaged 6.859. In checking the pH values of the groups of samples collected at different times of the year and therefore from animals under different feeding conditions no appreciable variation is noted. Monroe and Perkins (1) reported higher acid on rumen ingesta from cows on pasture than when roughages, corn, and A.I.V. silage were fed.

They also found when samples of ingesta were taken at two hour intervals, those taken prior to the morning feeding were the most alkaline. After feeding, the ingesta became more acid, with a trend toward greater alkalinity after several hours following the feeding period.

The same authors found the ingesta of animals which were slaughtered 12 to 18 hours after the last feeding to have a pH value of 7.34. This is somewhat more alkaline than the average of the samples reported herein. Undoubtedly many of the samples reported in our work were taken from cows which had not eaten any roughage for at least 12 hours before slaughter as it is customary to withhold feed from animals about 12 hours before slaughtering.

The data in table 2 indicate very definitely that the ingesta increased in alkalinity when it was allowed to remain in the laboratory at room temperature. In sample numbers 7, 8 and 9, the pH determinations covered a period of 6 to 7 days, with an appreciable increase in alkalinity.

The work of Monroe and Perkins (1) indicated an appreciable increase in acidity when the ingesta were incubated for 8 hours at 100° F.

No explanation is suggested for the ingesta becoming alkaline in the trials reported herein, on standing. If acid producing organisms were present in the ingesta in appreciable numbers the opposite would be expected. However, the data on a fairly large number of samples indicated that the samples of ingesta on standing were alkaline.

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TABLES AND NOMOGRAPH FOR SHARP AND HART'S EQUATION FOR THE CALCULATION OF TOTAL SOLIDS IN MILK

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It has long been known that the previous temperature history and manner of treatment received by milk has an influence upon its specific gravity as measured at 15° C. An important contribution towards a method to eliminate this influence was made by Bakke and Honegger (1). Their work was extended and corroborated by Hoyt, Lampert *et al.* (2). Lampert (3) also made a general review of this and other work pertaining to the use of the lactometer.

In a detailed study of the subject, Sharp and Hart (4) recommend that the specific gravity of milk be measured at 30° C., the milk previously having been warmed to 45° C. for one-half minute, thus insuring that the determination is made while the fat is in the liquid state. The following equation was evolved for the calculation of total solids from the fat content and specific gravity:

$$\text{Total solids} = 1.2537\ \text{fat} + 0.2680\ \frac{\text{lactometer}}{\text{sp.gr. of milk}}.$$

In 421 comparisons the deviation between calculated and gravimetric results did not exceed 0.30 per cent. This variation is comparable to that which may result when the ordinary Babcock procedure is used to calculate

TABLE 1

Fat factors for total solids in milk according to Sharp and Hart's equation

Per cent fat	Factor	Per cent fat	Factor	Per cent fat	Factor
0.1	0.1254	2.1	2.633	4.1	5.140
0.2	0.2507	2.2	2.758	4.2	5.266
0.3	0.3761	2.3	2.884	4.3	5.391
0.4	0.5015	2.4	3.009	4.4	5.516
0.5	0.6269	2.5	3.134	4.5	5.642
0.6	0.7522	2.6	3.260	4.6	5.767
0.7	0.8776	2.7	3.385	4.7	5.892
0.8	1.003	2.8	3.510	4.8	6.018
0.9	1.128	2.9	3.636	4.9	6.143
1.0	1.254	3.0	3.761	5.0	6.269
1.1	1.379	3.1	3.887	5.1	6.394
1.2	1.504	3.2	4.011	5.2	6.519
1.3	1.630	3.3	4.137	5.3	6.645
1.4	1.755	3.4	4.262	5.4	6.770
1.5	1.881	3.5	4.388	5.5	6.895
1.6	2.006	3.6	4.513	5.6	7.021
1.7	2.131	3.7	4.639	5.7	7.146
1.8	2.257	3.8	4.764	5.8	7.272
1.9	2.382	3.9	4.890	5.9	7.397
2.0	2.507	4.0	5.015	6.0	7.522

Received for publication January 11, 1941.

FIG. 1. Nomograph for total solids in milk according to Sharp and Hart's equation. Directions: A line or straight edge joining the lactometer reading on the left hand column with the per cent fat shown on the right hand column will pass through the center column at a point corresponding to the per cent total solids.

the total solids or solids-not-fat in milk. Inasmuch as the Sharp and Hart method has a theoretical advantage over the Babcock procedure it should be studied further. The increased length of time to prepare the sample and obtain a reading as well as the calculations needed may deter some from using the method.

Recently the writer (5) published a nomograph for finding the solids-not-fat according to the Babcock formula. Since it may facilitate at least part of the effort in using the Sharp and Hart method, the present tables and nomograph have been prepared. In table 1 factors for the fat content are given and in table 2 those for the lactometer readings, made at 30° C. A line on the nomograph joining the figures for the fat content and the lactometer reading for a given sample of milk will pass through the scale

TABLE 2

Lactometer factors for total solids in milk according to Sharp and Hart's equation

Lactometer reading at 30° C.	Factor	Lactometer reading at 30° C.	Factor
22.0	5.769	30.0	7.806
22.5	5.897	30.5	7.897
23.0	6.025	31.0	8.058
23.5	6.153	31.5	8.184
24.0	6.281	32.0	8.310
24.5	6.408	32.5	8.436
25.0	6.537	33.0	8.561
25.5	6.664	33.5	8.687
26.0	6.791	34.0	8.812
26.5	6.918	34.5	8.938
27.0	7.046	.....	.....
27.5	7.173	.....	.....
28.0	7.300	.....	.....
28.5	7.426	.....	.....
29.0	7.553	.....	.....
29.5	7.679	.....	.....

DIRECTIONS.—To find the total solids according to the equation $1.2537 \text{ fat} + 0.2680 \frac{\text{lactometer at 30° C.}}{\text{Sp. Gr.}}$ add the factor corresponding to the fat content in table 1 to the factor for the lactometer reading found from table 2. Example—Fat = 3.8%, Lactometer 30.5. The fat factor is 4.764, the lactometer factor is 7.897. The sum of the factors is 12.661 or the total solids content is 12.66%.

at a point corresponding to the total solids content. In making the calculations all significant figures in the Sharp and Hart equation were used. The tables will give figures to the nearest one one-hundredth per cent and the nomograph can be read to within five one-hundredths as per cent total solids.

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THE EFFECT OF PASTEURIZATION ON *ESCH. COLI* IN MILK AND ICE CREAM MIX

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For the pasteurization of ice cream mixes a temperature higher than that used for milk is generally employed. The reason for this is based partly on an improvement which results in certain physical properties of the mix and partly on the belief that *Esch. coli*, which is often considered a test organism, is more resistant to pasteurization when suspended in an ice cream mix than when contained in milk. While there is experimental evidence establishing the protective action of mix, at least for certain strains of *coli*, there are a number of questions dealing with the phenomenon which have been left unanswered, which concern particularly the nature of the protective action and the substances present in the mix which may be responsible for the increased resistance of the organism. The present study was undertaken to answer these questions, if possible, by means of quantitative data on the comparative resistance of *Esch. coli* in milk and ice cream mix.

A review of the literature reveals that but one article deals with experimental data on the comparative resistance of *Esch. coli* in the two media to the pasteurization process as ordinarily practiced. In this report, by Fabian and Coulter (1), the results of tests on forty-four strains of *coli* and aerogenes are described. Cultures of each strain were inoculated into ice cream mix and skim milk, held for two hours at 37° C. and then pasteurized. Subcultures were made into lactose broth and the success or failure of the pasteurization process was indicated by a lack of growth or growth of surviving coli in this media. At 144.5° F. no strains survived in milk. In ice cream mix the results were variable, in different experiments as few as two and as many as ten of the 44 cultures tested survived. While no data are given in the report on the number of organisms at the start of heating, it appears from the number inoculated that the strains which survived were of unusual resistance. It is likely also that the ice cream mix cultures contained greater numbers of organisms than the milk cultures. These authors also studied the individual ingredients of the ice cream mix but were unable to demonstrate a protective action which could be ascribed to any one ingredient. Mention of this point is important inasmuch as their paper has on a number of occasions been quoted as demonstrating the protective action of sugar. In general, while these experiments seem to show that an ice cream mix exerts a protective action on certain strains of the colon bacillus, they offer no suggestions as to the cause of the protective action. In addi-

Received for publication January 13, 1941.

tion they do not show whether there is a general increase in the resistance of the organisms or whether the apparent increase in survival is due to some other cause such as the preservation of clumps by the high viscosity of the mix.

In addition to the studies of Fabian and Coulter, there are a number of publications dealing with the protective action of hypertonic sugar solutions. These, which will be discussed below, have for the most part been made at temperatures lower than those which are usually used for pasteurization or with stronger sugar solutions than are used in practice and therefore do not bear directly on the problem under discussion.

In our own studies the possible protective action of ice cream mix was studied in the following way. Samples of milk and commercial ice cream were heated momentarily to 100 degrees centigrade, then cooled rapidly in order to destroy any *coli* organisms already present and to reduce to a minimum the numbers of other organisms. Three ounce quantities of milk or mix in four ounce bottles were then inoculated with *coli* cultures and allowed to incubate at 37° C. for three or four hours. The *coli* culture used was a 24 hour old culture of a human strain recently isolated. At the end of the incubation period, the bottles were placed in a deep water bath regu-

TABLE 1

Survival of Esch. coli in milk and ice cream mix at 143° F.

Experiment number	No. organisms before heating	No. of survivors after				
		10 min.	20 min.	25 min.	30 min.	35 min.
A. Milk						
1	1,000,000 (2.6)	0.0	0	0		
2	" (2.7)	3.7	0	0		
3	" (0.2)	450.0	0	0		
4	" (0.7)	0.0	0	0		
5	" (2.0)	0.0	0	0		
6	" (0.7)	29.0	0	0		
7	" (1.1)	0.0	0	0		
8	" (0.9)	0.0	0	0		
Average	1,000,000 (1.4)	60.4				
B. Ice Cream Mix						
1	1,000,000* (2.6)	6400.0	1500.0	170.0	18.0	35.0
2	" (2.2)	7700.0	0.4	0.0	0.4	0.4
3	" (0.5)	120000.0	1600.0	36.0	0.0	0.0
4	" (1.8)	890.0	0.6	1.2	2.4	1.2
5	" (16)	5100.0	180.0	5.0	0.3	0.0
6	" (1.0)	2100.0	14.0	2.0	1.0	1.0
7	" (0.6)	1100.0	33.0	0.0	0.0	0.0
8	" (0.5)	44.0	2.0	0.0	0.0	0.0
Average	1,000,000	18000.0	416.0	27.0	2.8	0.2

* The data have been calculated on the basis of an initial one million organisms. The actual counts in millions are given in parentheses.

lated at 143° F. The milk attained the temperature of the bath in approximately six minutes; the bottles of mix in about seven minutes. Counting from the time at which the temperature of the materials reached 143° F., one-ml. quantities of milk or ice cream mix were withdrawn at suitable intervals, cooled by diluting in 99 ml. of sterile tap water and plated in this and higher dilutions on eosin-methylene-blue agar. After 48 hours of incubation at 37° C. the *coli* colonies were counted. The largest quantity of undiluted material which was plated out was 0.01 ml. and the results were calculated on the basis of this volume. However, the figures in the table may be read as applying to any other unit of volume. Thus, referring to Experiment 1 of the ice cream series, an initial 1,000,000 organisms would yield 6400 survivors whether the original organisms were contained in 0.01 ml. or in 1 ml.

The results of eight tests on ice cream mix with the corresponding control tests on milk are given in table 1.

The data in the table have too wide a variation as between experiments for any degree of refined mathematical analysis of the average values. They do show, however, very clearly that commercial ice cream does exert a protective action for *Esch. coli*. Inspection of the figures in the table indicates that the same percentage reduction in the number of living organisms requires about twice as long in ice cream as is required in milk. The fact that in each experiment in ice cream mix there is a progressive reduction of numbers of survivors, with time, strongly suggests that the general level of resistance of all the organisms present is raised rather than that of a few in the suspension. This fact in turn argues against the chance that the end result is due to the survival of organisms buried in clumps.

Having confirmed the fact that commercial ice cream mix protects *coli* against the action of heat, there remained the search for the individual ingredient which might be responsible for the protective effect. It was assumed that some ingredient would be responsible, although the possibility was recognized that certain conditions of colloid structure, rather than the presence of a single substance might ultimately be found to underlie the phenomenon under investigation.

Proceeding on the first assumption, experiments were run in which single ingredients of ice cream were added to milk, *coli* organisms added, and the mixture pasteurized at 143° F. The results are summarized in table 2.

It will be noted that butter, flavor, color, sugar (liquid or granulated), and gelatin were without influence on the survival of *Esch. coli* at the pasteurization temperature. On the other hand, sodium alginate and locust bean gum very clearly increased the apparent resistance of the organism to the pasteurization temperature. The degree of protection is approximately that observed in the commercial ice cream, although the effect may be enhanced by the presence of other ingredients. A possible objection to the experiments just cited lay in the fact that the materials were dissolved in

TABLE 2

Influence of individual ingredients of ice cream on the survival of Esch. coli in milk at 143° F.

Added ingredients	Experiment number	Colony count before heating	Number of survivors per initial million after exposures of				
			10 min.	20 min.	25 min.	30 min.	35 min.
Butter	1A	1,000,000 (17)	23	0	0	0	0
	1B	" (9)	11	0	0	0	0
	2A	" (19)	0	0	0	0	0
Flavor	2B	" (6)	33	0	0	0	0
	2C	" (10)	0	0	0	0	0
	3A	" (14)	7	0	0	0	0
Color	3B	" (23)	0	0	0	0	0
	3C	" (14)	0	0	0	0	0
Granulated sugar	4	" (8)	25	0	0	0	0
Liquid sugar	5	" (40)	16	0	0	0	0
Sodium alginate*	6	" (25)	2500	5.0	0.75	0.25	0
Gelatin	7	" (14)	7	0	0	0	0
Locust bean gum*	8	" (19)	4700	11.0	6.0	1.0	0

* 0.5% final concentration.

milk, without an adjustment of pH. Sodium alginate tends to make solutions alkaline which generally increases resistance, whereas our gelatin and locust bean gum reacted acidic, thus favoring the action of heat. In order to study this pH effect another series of experiments was run, adding the sodium alginate, locust bean gum and gelatin to water and to buffer at 6.7. The results are summarized in table 3.

TABLE 3

Survival of Esch. coli at 143° F. in solutions of stabilizers at different pH

Stabilizer	pH of solution	No. organisms before heating	Number of survivors per initial million organisms after exposures of				
			10 min.	20 min.	25 min.	30 min.	35 min.
Gelatin	4.8	1,000,000 (9)	0	0	0	0	0
	6.7	" (16)	6.3	0	0	0	0
Locust bean gum	4.3	" (9)	145	0.6	0	0	0
	6.7	" (19)	4800	11	6	1.1	0
Sodium alginate	8.1	" (6)	2700	50	0	3	0
	6.7	" (18)	330	1.7	0.5	0	0
Buffer control	8.0	" (19)	0	0	0	0	0
		(3)	0	0	0	0	0

The gelatin used gave a pH in water of 4.8. At this pH and at pH 6.7 the killing was approximately as rapid as in milk or in buffer alone. Locust bean gum, which gave a pH of 4.3 in water showed a somewhat greater survival at 6.7 than at 4.3. Sodium alginate, on the other hand, which unbuffered yielded a solution of 8.1, was not quite so effective at 6.7 as at this pH but still offered a considerable protection to the organisms suspended in

its solution. These results show that in commercial ice cream mix as prepared at present the substances which protect the *coli* organism against the action of the temperature of 143° F. are the stabilizers, locust bean gum and sodium alginate.

As a further test of the necessity of the stabilizers, an ice cream mix was prepared in the laboratory according to a commercial formula, with milk, condensed milk, heavy cream and granulated sugar. At 143° F. per 1,000,000 original organisms, less than 16 survived ten minutes; none survived as long as twenty minutes. The killing action in this medium therefore closely approximated that in pure milk.

DISCUSSION

Our experimental findings show the peculiar protective action of sodium alginate and locust bean gum for *Esch. coli* against the effects of heat, and also indicate that an ice cream mix without these substances is no more difficult to pasteurize than milk. The positive results of Fabian and Coulter were apparently obtained in the absence of both stabilizers which we have found responsible for protection. In seeking an explanation for this discrepancy a number of points may be considered. At 143° F. Fabian and Coulter find that only certain strains appeared to be more resistant in ice cream mix than in milk. In addition, they incubated their cultures for a period of two hours with their ice cream mix, which has been found by Fay (2) considerably to increase the resistance of *coli* to heat. The period of incubation may also have increased the differences in the counts of milk and ice cream mix suspensions so that actually more organisms per unit volume were heated in one case than in the other. Other conditions being equal, a longer time would be required to produce the same degree of sterilization for the suspension containing the larger number of organisms.

A number of workers have found that hypertonic solutions of sugar increase the resistance of *Esch. coli* and other organisms to heat. The literature has been reviewed by Fay. Of particular interest are the observations of Robertson (3) who found that a culture of *Esch. coli* was more resistant to pasteurization at 143.6° F. in 23.1 per cent sucrose than in a 1 per cent solution of this sugar. With higher concentrations of sugar progressively greater degrees of resistance were noted. At the present time the concentration of sucrose in ice cream mix rarely exceeds 15 per cent so that it can only be inferred that an increased resistance would have been demonstrated in ice cream mix. Fay, working with approximately 15 per cent sucrose found that the resistance of *coli* was increased on the average of 2 5 times by this concentration. The experiments were conducted, however, at 130° F. with an exposure of nine minutes. It cannot be assumed that the same degree of increased resistance would be effective at higher temperatures. Fay has shown that the protective action of sugar against the coagulation

of egg white falls off very rapidly with increase in temperature and it is reasonable to assume that such should be the case with organisms. In each instance the protective action is probably connected with the dehydrating and water-binding properties of strong sugar solutions—both dried organisms and dried proteins being markedly heat resistant. As the temperature of such hypertonic solutions is raised, more and more free water is produced in the mixture until at some point the unbound water occurs in such proportion that the reaction with protein goes on essentially at the rate at which it would without sugar present. Sodium alginate and locust bean gum might well work in the same manner as sugar by binding water and it would be expected that with higher temperatures the protective action of these stabilizers would become less, becoming undiscernible above some given temperature as yet undetermined. Whether or not the temperature of 155° F. is safely above this temperature is a point which will require further investigation, but from the experience of one of us (C.P.) in the practical testing of ice cream mixes, it would appear that the general absence of *coli* from pasteurized ice cream mixes, other conditions being satisfactory, indicates that this temperature is above the critical one for the stabilizers in common use at the present time. Fabian and Coulter in their experiments with various strains of *coli* found none which survived 155° F. for thirty minutes.

A point worthy of consideration is the effect of the protective substances on other organisms. While our own work was limited to *Esch. coli* the possibility is suggested that other organisms may show a similar heat resistance in the presence of certain stabilizers. Oldenbusch and co-workers (4) found a slight increase of resistance when the survival of staphylococcus, typhoid, and streptococcus was compared in milk and ice cream mix. While this difference would appear to be significant these authors concluded that it was within the limits of their experimental error. Robertson noted increased resistance for *Streptococcus thermophilus, Escherichia coli, Micrococcus aureus,* and *Sarcina lutea* in the presence of 23 or more per cent of sugar. Fay, working with sugar solutions at a lower temperature and for a shorter time than is ordinarily used in pasteurization, found that in the presence of sugar some organisms show increased resistance while others were uninfluenced. *Staphylococcus aureus* and *Aerobacter aerogenes* belonged to the former group. While further work is needed before generalizations can be made it would appear not unreasonable to assume that some organisms, including possibly some pathogens, might, with *Esch. coli,* constitute a group whose resistance to heat is increased in the presence of certain substances.

SUMMARY AND CONCLUSIONS

1. Comparative studies on milk and commercial ice cream show that the latter exerts a protective action for *Esch. coli* against the pasteurization

temperature of 143° F. The apparent resistance of the organism is approximately doubled.

2. In commercial ice cream the substances responsible for this protection are the stabilizers, locust beam gum and sodium alginate. Sugar and gelatin were found to exert no protective effect.

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EFFECT OF FREE FAT ACIDS OF MILK FAT ON CURD TENSION OF MILK. RELATION TO MILK ESTERASE, TEMPERATURE, USE OF $CaCl_2$, KIND OF FAT ACID, MILK LIPASE AND CHURNING*

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In a previous paper (1) presented jointly from this Division and the Division of Dairy Industry, University of California, preliminary evidence was given that raw skim milk will hydrolyze diglycol laurate and that this prevents the normal clotting of milk by rennet at 35° C. We are presenting in this paper the experimental evidence obtained in this laboratory in explanation of this phenomenon as well as further studies regarding various other related factors which may adversely affect the clotting properties of milk or which may overcome this tendency.

EXPERIMENTAL

General Procedures and Methods. Dispersion of esters and fat acids employed in the various studies was effected by vigorous mechanical agitation for 30 minutes, whenever possible at a temperature above the melting point of the dispersed substance. When temperature control was necessary in any experiment or part thereof, an electrically heated constant temperature water bath was employed. A glass electrode pH meter was used almost exclusively for measuring acidity. Curd tension was obtained in some cases by means of the Hill knives and spring balance and in other by the Submarine Signal Company curd tension meter.

1. *Evidence for enzymatic hydrolysis of diglycol laurate by milk.* It seems proper to refer to such an enzyme as an esterase since a simple fat acid ester is the substrate. It is probably one of the "lipases" of milk studied recently by Krukovsky and Sharp (2, 3), Herrington and Krukovsky (4, 5, 6), Mattick and Kay (7), and others. The conditions favorable for the liberation of lauric acid from diglycol laurate by raw milk and milk products resemble closely those developed by these workers for demonstrating lipolysis in raw milk. Typical experiments which collectively show that a milk enzyme decomposes diglycol laurate are summarized in table 1. Apparent variations in extent of hydrolysis are due to the work having been done at different times on different samples of milk. The increase in acidity, measured chiefly as pH, occurred at 10° C. or lower, except in experiment 8.

The data show (a) that the lauric acid ester is hydrolyzed in raw skim

Received for publication January 17, 1941.

* Paper No. 1877, Scientific Journal Series, Minnesota Agricultural Experiment Station.

TABLE 1

Change in pH various products containing added diglycol laurate, indicating enzymatic hydrolysis of the ester

Exp. no.	Kind of product	Proportion of product to ester		Aging temp.	pH		
		Product	Ester		Immediately	1st period	2nd period
				°C.			
1	Raw skim	100	0.0	10	6.60	6.62 (36)[a]	6.60 (49)
	Raw skim	100	0.5	10	6.65	6.28 (36)	6.18 (49)
2	Raw rennet serum[b]	100	0.0	1	6.59	6.58 (18)	6.57 (42)
	Raw rennet serum	100	0.5	1	6.65	6.52 (18)	6.42 (42)
3	Raw rennet serum ultra filtrate[c]	100	0.0	1	6.59	6.60 (18)	6.58 (42)
	Raw rennet serum ultra filtrate	100	0.5	1	6.65	6.57 (18)	6.54 (42)
4	Raw acid serum ultra filtrate[d]	100	0.0	1	6.57	6.59 (15)	6.59 (40)
	Raw acid serum ultra filtrate	100	0.5	1	6.61	6.62 (15)	6.62 (40)
5	2.5% Ca phosphocaseinate sol	100	0.0	10	6.50	6.53 (36)	6.53 (49)
	2.5% Ca phosphocaseinate sol	100	0.5	10	6.65	6.65 (36)	6.63 (49)
6	2.5% Ca caseinate sol	100	0.0	1	7.13	7.13 (20)	7.13 (44)
	2.5% Ca caseinate sol	100	0.5	1	7.20	7.18 (20)	7.12 (44)
7	Egg white sol[e]	100	0.0	1	6.77	6.87 (16)	6.95 (40)
	Egg white sol	100	0.5	1	6.75	6.98 (16)	7.05 (40)
8	Raw skim[f]	2	0.5	38	7.70	7.38 (16)[g]	.....
	Pasteurized skim	2	0.5	38	7.75	7.63 (16)[h]	.....

[a] The numbers in parentheses indicate the number of hours the sample was held.

[b] Prepared by rennet coagulation of raw skim milk at 40° C., serum centrifuged and filtered through paper on Büchner funnel, using suction.

[c] Same as Exp. 2 except filtered by suction through a Berkfeldt filter.

[d] N/10 HCl was added to raw skim milk to pH 4.6 and the acid serum filtered through Berkfeldt filter and filtrate adjusted to pH 6.6 using N/10 NaOH.

[e] Raw egg white was diluted with 9 volumes distilled water; 20% M/1 NaCl added to disperse the globulin and the pH adjusted from 8.1 to 6.77 using 0.6% H_3PO_4 solution.

[f] In this experiment one ml. skim milk was added to 50 ml. 0.5% colloidal emulsion of diglycol laurate + one drop formalin.

[g] The titratable acidity of this sample increased 100 per cent.

[h] The titratable acidity of this sample increased 22 per cent.

milk and in rennet whey from raw milk; (b) that the hydrolysis is brought about by small amounts of raw skim milk on incubation in the presence of formaldehyde but not by pasteurized skim milk; (c) that the catalytic agent is largely, if not completely, removed from raw rennet whey and raw acid serum by a coarse ultrafilter (Berkfeldt filter); and (d) that the catalytic agent is not present in raw egg white sol, synthetic calcium caseinate sol or synthetic calcium phosphocaseinate sol.

2. *Relation of temperature, lauric acid and use of $CaCl_2$ to pH and curd tension of milk treated with diglycol laurate.* It was shown in the previous paper (1) that the conditions found by Tarassuk for demonstrating the

impaired rennet clotting properties of raw skim milk to which diglycol laurate has been added are (a) proper dispersion of the ester into the milk at room temperature, (b) aging the emulsion at low temperature, (c) warming the emulsion to clotting temperature. Having discovered that hydrolysis of the ester by milk esterase is the cause of the decrease in pH during the period of aging at low temperature, experiments were conducted to compare the clotting ability of ester treated milk with that of milk to which free lauric acid was added. Several experiments of this kind were carried out, but only typical results will be reported. In all cases sufficient lauric acid was vigorously emulsified in the milk for 30 minutes at 45° C. (this is slightly above the melting point of the acid) to lower the pH to that attained in the non-clotting, ester-treated milk after aging. The experiments also included a study of the favorable effect of adding $CaCl_2$ in the curd tension test, an effect already observed by Tarassuk (1).

In the course of these studies we found that the free lauric acid added to the milk would impair the clotting ability only after aging.[a] Milk containing freshly emulsified lauric acid exhibits normal curd tension.[a] Evidently the gradual decrease in curd tension shown by milk containing added diglycol laurate at low temperature is not wholly due to the time necessary for the liberation of lauric acid. The primary purpose of the low temperature was to prevent lactose fermentation, but it seems very probable from later observations that the same effects could be produced at temperatures perhaps as high as 20–25° C. if lactic acid development could be excluded. However, a bacterial antiseptic would have to be used which does not in itself interfere with normal clot formation by rennin. We did find that neither milk in which lauric acid was liberated from the ester nor the milk to which the acid was added would show impairment of clotting ability if the curd tension test is performed at 40° C. instead of at the normal temperature of 35° C. or if the milk is first heated momentarily to 50–55° C. before bringing it to 35° C. for the curd test.

Results bearing on several of these conclusions are shown in table 2. Experiments II, III and IV were performed simultaneously on the same lot of raw skim milk. Attention is directed to the markedly similar results obtained from the hydrolyzed lauric acid ester and the added lauric acid. Experiment IV shows that the effects of the acid are completely nullified at 35° C. if $CaCl_2$ is used in the curd test and that this is associated with a rather marked further decrease in pH. Experiment II shows that complete loss of clotting ability is attained more rapidly when the lauric acid is added directly to the milk. This would be expected.

Experiments I, II and III, table 2 show how greatly the effects of both

[a] It has subsequently been observed that some samples of milk do exhibit a substantial reduction in curd tension immediately after addition of lauric acid. The divergent results are being investigated.

TABLE 2

Comparative effects on curd tension and pH of hydrolysis of diglycol laurate and added lauric acid and relations thereto of temperature and $CaCl_2$

Sample no.	Description of sample	Curd tension[a]			pH		
		A	B	C	A	B	C
		gm.	*gm.*	*gm.*			
	Experiment I (40° C.)						
1	Raw skim + 0.5% dg.l.[c]	68	50 (24)[b]	78 (48)	6.65	6.47	6.42
2	Raw skim + 0.3% l.a.[d]	104	74	.....	6.18	6.30	.....
	Experiment II (35° C.)						
3	Raw skim + 0.5% dg.l.	63	17 (24)	0 (48)	6.48	6.38	6.37
4	Raw skim + 0.3% l.a.	.....	4	.....	.....	6.32	.....
	Experiment III (35° C.)						
5	Raw skim + 5% dg.l. △ 55° C.[e]	.....	76 (24)	.....	.....	6.38	.....
6	Raw skim + 0.3% l.a. △ 55° C.	.....	68	.....	.....	6.32	.....
	Experiment IV (35° C.)						
7	Raw skim + 0.5% dg.l. + $CaCl_2$	70	83 (24)	88 (48)	5.75	5.68	5.67
8	Raw skim + 0.3% l.a. + $CaCl_2$	.....	103	.....	.....	5.60	.....

[a] Determined by Hill knives at temperature indicated after number of each experiment, using one ml. concentrated rennet extract to 100 ml. milk. When $CaCl_2$ was used, as in Exp. V, 2.5 ml. Hill $CaCl_2$ sol. were first added to 100 ml. of milk. Data represent average of duplicate determinations.

[b] The numbers in parentheses indicate the number of hours the samples for that experiment were aged. Only subsamples B and C were aged at low temperatures, the data in column A being obtained on the freshly prepared emulsions.

[c] dg.l. = diglycol laurate. [d] l.a. = lauric acid.

[e] The figure after △ indicates the temperature to which the sample was heated momentarily before bringing it to 35° C. for the curd tension test.

added and liberated lauric acid are modified by the clotting temperature employed or by flash heating to 50–55° C. Since the melting point of lauric acid lies between 40° C. and 50° C.,[1] the results do not seem to be related to the physical condition of the acid. The duration of holding the samples at 40° C. was not controlled in Experiment I, which might account for the somewhat variable curd tensions obtained at the different periods. Therefore, it seemed of interest to determine the rapidity with which a lauric acid treated milk, aged in the cold, would regain its normal clotting property at 40° C. and also whether the same result would follow if the milk were held for a sufficiently long time at 35° C. A typical experiment is shown in table 3. The curd tests in this case were made at 35° C. without $CaCl_2$, using the Submarine Signal Company curd tension meter.[2] The milk was raw market skim milk. Undiluted rennet extract was added at the rate of 1 ml. per 150 ml. milk. It is evident that normal curd tension of lauric acid treated milk which has been aged in the cold may be restored within one-half hour at 40°

[1] The lauric acid used had a melting point of 44° C., which was reduced to 42.5° C. by emulsifying in water or whey.

[2] A comparison of the curd tension of a number of samples of normal milk by the Hill knives with the surface cut and body cut values given by the Submarine Signal Company curd tension meter showed that the Hill value always corresponded most closely with the surface cut value.

C. and that there is also considerable restoration of normal clotting properties at 35° C. The question whether this will ever be complete at the latter temperature is not satisfactorily answered in the experiment reported since the result with the five-hour sample is complicated by a drop in pH which undoubtedly assisted the restoration of curd tension. However, in another experiment at 35° C., not reported here in detail, in which the pH did not change, only about 75 per cent of the original curd tension had been restored at the end of six hours.

TABLE 3

Restoration of curd tension determined at 35° C. of aged, lauric-acid-treated skim milk, by conditioning at 35° C. and 40° C., respectively

Description of sample	Temp. of conditioning	Time of conditioning	Curd tension*		
			Surface	Body	pH
	°C.	hr.	gm.	gm.	
Fresh emulsion	35	0.0	50	39	6.28
Aged emulsion	35	0.0	0	0	6.30
Aged emulsion	35	0.5	3	8	6.33
Aged emulsion	35	1.0	14	22	6.30
Aged emulsion	35	2.0	18	22	6.28
Aged emulsion	35	5.0	50	37	6.18
Aged emulsion	40	0.0	3	10	6.33
Aged emulsion	40	0.5	50	43	6.33
Aged emulsion	40	1.0	53	45	6.30

* Submarine Signal Company curd tension meter values.

3. *Restoration of normal clotting by addition of $CaCl_2$.* A direct effect of lauric acid on rennin itself first had to be ruled out before the effect of $CaCl_2$ could be studied more closely. To this end the clotting property of two portions of commercial rennet extract[3] was compared after they had been treated as follows. Portion A, warmed to 40° C., was diluted with 25 per cent melted lauric acid and the mixture emulsified for 30 minutes at this temperature. The ratio of rennin to lauric acid in this emulsion is approximately that found when adding one ml. rennet extract to 100 ml. milk containing 0.3 per cent lauric acid. Portion B was treated similarly with water. Graded amounts of the two portions were then added to 100 ml. portions of raw skim milk at 35° C., and their curd tensions determined by the Hill knives without adding $CaCl_2$. The results are tabulated at top of p. 434.

The evidence is slight, if any, that the contact of the rennin with the concentrated emulsion of lauric acid affected its activity under the conditions of the experiment.

The nullifying effect of $CaCl_2$ on the action of lauric acid was first examined from the standpoint of a chemical reaction occurring between the salt and the acid. This was determined indirectly through the effects on

[3] The rennet extract used throughout this study was kindly furnished by Chr. Hansen's Laboratory, Inc., Milwaukee, Wis.

Amount rennet extract added per 100 ml.	Curd tension when using	
	Lauric acid treated rennet A	Water treated rennet B
ml.	*gm.*	*gm.*
1.3	58	67
1.0	68	72
0.7	62	62
0.4	45	55

curd tension rather than by isolating the products of such a reaction. For this purpose two portions of raw skim milk at 45° C. were subjected to a 30-minute mechanical emulsification respectively with (A) 0.3 per cent lauric acid and (B) 0.25 per cent Hill $CaCl_2$ reagent[4] which had been heated at 80° C. for five minutes with 10.7 per cent lauric acid. The latter mixture contained $CaCl_2$ and fat acid in chemical equivalent (approximately) amounts. Both portions were aged for 24 hours at 0–5° C. and their curd tension obtained at 35° C. by the Hill knives. Portion A had zero curd tension, portion B a curd tension of 73 grams. This result indicates that a product is produced by allowing $CaCl_2$ and lauric acid to react chemically which exerts no effect on the clotting of milk with rennin.

The question was next examined whether the low pH of $CaCl_2$-treated milk, to which attention was called previously in connection with Experiment IV, table 2, plays a role in nullifying the effects of lauric acid. It is difficult to devise an experiment which will give an unequivocal answer to this question. Any acid which lowers the pH of milk to the acidity found in the experiment cited will itself increase the calcium available for combining with fat acid. However, it was hoped that some light might be thrown on the question by comparing the restoration of curd tension in milk rendered non-clotting through added lauric acid (a) when the pH only was reduced and (b) when a rather large increase in calcium concentration was produced without changing the pH of the non-clotting lauric acid milk. A 1.0 N solution of HCl was used for the pH adjustment and mixtures of the Hill $CaCl_2$ reagent and calcium sucrate solution[5] were used to increase the calcium content without changing the pH. The effect of these reagents was tested on two portions of the same lot of milk, one portion of which was emulsified for 30 minutes at 45° C. with 0.3 per cent lauric acid and aged for 24 hours at 0–5° C., and the other portion, which served as

[4] Calcium chloride is usually regarded as an acid compound because of its pH reducing effect when added to milk. However, it does not seem to be generally recognized that aqueous solutions of $CaCl_2$ are slightly alkaline, showing that the salt has a greater tendency to form $Ca(OH)_2$ than HCl in water. The pH of the Hill curd tension $CaCl_2$ reagent is 7.7, which on dilution (2.5 ml. to 100) with distilled water rises to 8.1.

[5] The calcium sucrate was prepared by saturating a 33 per cent sucrose solution with $Ca(OH)_2$, and filtering off the surplus lime.

control, treated in the same way without the addition of lauric acid. Curd tensions were determined at 35° C. by the Hill knives.

The results of this experiment are given in table 4. They indicate that the increase in calcium salts obtained either by direct addition or indirectly through an increase in acidity are probably more important in overcoming the detrimental effects of lauric acid than the increase in acidity. However, the higher curd tension shown by the control milk when HCl was added than when the calcium content only was increased suggests that a pH effect is not entirely negligible.

TABLE 4

Relative effects of calcium salts and decreased pH in restoring normal clotting properties to milk containing added lauric acid

Sample no.	Reagent added per 100 ml.	Control milk		Lauric acid milk	
		Curd tension	pH	Curd tension	pH
		gm.		*gm.*	
1	None	51	6.70	8	6.32
2	2.5 ml. Hill $CaCl_2$	55	5.85	85	5.58
3	1.5 ml. 1.0 N. HCl*	77	5.88	60	5.55
4	0.5 ml. Hill $CaCl_2$ + 0.28 ml. calcium sucrate	54	6.65	79	6.30
5	1.0 ml. Hill $CaCl_2$ + 0.56 ml. calcium sucrate	37	6.69	56	6.30

* The HCl solution was added to the cold milk by capillary tube inserted into the milk which was being mechanically stirred.

4. *Effects of other fat acids of milk fat on clotting of milk by rennin.* All of the other commonly recognized fat acids of milk fat from C_4 to C_{18}, inclusive, except myristic acid, were available for study. However, ethyl myristate[6] (b.p. 137.5° C. at 2 mm.) was tested in an experiment in which the activity of the milk esterase was depended upon, as was likewise done in the case of butyl stearate and diglycol oleate, which were also tested. The oleic acid used was a sample of high purity[7] (I_2 value 88.3). The other fat acids were of commercial origin and were probably reasonably pure products, inasmuch as they showed essentially normal physical properties.

Raw skim milk of market origin was used in order to have milk of as uniform curd tension as possible since the various fat acids could not conveniently be tested in the same milk. The raw market skim milk used was found to give quite uniform curd tensions from day to day. When a free fat acid was to be tested a preliminary experiment was usually run to determine the amount of acid required to reduce the pH to 6.2–6.3 under the

[6] This was supplied through the courtesy of Drs. W. H. Lauer and F. L. Greenwood of the Division of Organic Chemistry, Institute of Technology, University of Minnesota.

[7] This was supplied through the courtesy of Dr. G. O. Burr of the Department of Botany, University of Minnesota.

conditions of the experiment, namely, vigorous agitation for 30 minutes at 45° C. The agitation procedure was carried out even when the acid dissolved readily in the milk, as in the case of butyric acid. When the emulsifying temperature was below the melting point of the acid a few tenths per cent of the melted acid were added slowly to the milk at 45° C. during the agitation, with the hope that there would be sufficient dispersion and ionization to bring about the desired increase in acidity. The result was not very successful in the case of palmitic and stearic acids, as shown by the data. In any case whether the fat acid increased the acidity of the skim milk or not, the curd tension at 35° C. and pH of the freshly prepared emulsion were determined (and in some cases also that of a control which had been agitated but to which no acid had been added) and the same properties determined again after 24 hours and sometimes also after 48 hours aging at low temperature. Whenever the added acid showed an effect on the curd tension at 35° C. an effort was made to determine whether the effect could be overcome by holding the milk at 35° C. or 40° C., and also the holding period necessary to bring this about. When the fat acid esters were employed, 0.3 per cent emulsions of the esters were prepared and the experiments carried out in all other respects like those involving the lauric acid ester.

TABLE 5

Lack of effect of various fat acids of milk fat on curd tension of raw skim milk

Product added	Concentration used	Curd tensions						pH		
		Fresh		24 hr.		48 hr.		Fresh	24 hr.	48 hr.
		Surface	Body	Surface	Body	Surface	Body			
	per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*			
Butyric acid	0.1	63	48	63	51	.....	.....	6.20	6.20	.....
Caproic acid	0.15	71	48	72	49	.....	.....	6.05	6.10	.....
Caprylic acid	0.1	73	46	78	55	.....	.....	6.30	6.32	.....
Caprylic acid	0.3	93	73	74	64	.....	.....	5.90	6.02	.....
Ethyl myristate	0.3	51	38	55	43	54	44	6.55	6.32	6.30
Control	0.0	54	38	60	39	55	40	6.55	6.45	6.43
Palmitic acid	0.3	43	33	39	34	.....	.....	6.55	6.58	.....
Stearic acid	0.4	46	33	45	33	.....	.....	6.65	6.52	.....
Butyl stearate	0.3	29	24	35	28	42*	34*	6.65	6.55	6.35

* After holding this sample for one hour at 40° C. it showed curd tension readings at 35° C. of 53 (S), 38 (B) and a pH of 6.30 suggesting that the butyl stearate itself impairs the clotting properties and that this effect is gradually overcome by hydrolysis of the ester, which is accelerated by holding at 40° C.

Table 5 gives the results of the various experiments in which no effect of the fat acid could be discerned. The Submarine Signal Company curd tension meter was used and both surface-cut and body-cut values recorded. Only in the case of butyl stearate was any effect noticeable and, as explained in the footnote to the table, this cannot be attributed to the stearic acid.

TABLE 6

Effect of capric acid on curd tension and restoration of normal clotting properties at 35° C. and 40° C.

Description of sample	Temp. of conditioning	Time of conditioning	Curd tension		pH
			Surface	Body	
	°C.	hr.	gm.	gm.	
Fresh emulsion	35	0.0	50	45	6.20
Aged emulsion	35	0.0	0	0	6.20
Aged emulsion	35	0.5	0	0	6.18
Aged emulsion	35	1.0	0	0	6.18
Aged emulsion	35	2.0	3	5	6.20
Aged emulsion	35	5.0	40	29	6.12
Aged emulsion	40	0.0	0	0	6.20
Aged emulsion	40	0.5	29	31	6.18
Aged emulsion	40	1.0	50	43	6.20

Table 6 shows the results obtained with capric acid. Crystalline acid from Eastman Company, melting at 31° + C., was used. The data are very similar to those reported for lauric acid in table 3, the only difference being the less rapid restoration of the clotting of the capric acid milk on temperature conditioning at 35° C. and 40° C.

TABLE 7

Comparative effects on curd tension of oleic acid and diglycol oleate. Further effects of aging and hydrolysis at low temperature and effectiveness of temperature conditioning on restoration of normal clotting properties

Product added	Concentration	Temp. of conditioning	Time of conditioning	Time of low temp. aging	Curd tension		pH
					Surface	Body	
	per cent	°C.	hr.	hr.	gm.	gm.	
None (Control)	0.0	35	0	24	49	37	6.60
Oleic acid	0.4	35	0	0	6	6	6.28
Oleic acid	0.4	35	0	24	0	0	6.22
Oleic acid	0.4	35	3	24	16	15	6.02
Oleic acid	0.4	40	1	24	11	12	6.22
None (Control)	0.0	35	0	24	46	33	6.60
Diglycol oleate	0.3	35	0	0	11	14	6.60
Diglycol oleate	0.3	35	0	24	7	12	6.55
Diglycol oleate	0.3	35	0	48	10	13	6.40
Diglycol oleate	0.3	35	3	48	31	25	6.35
Diglycol oleate	0.3	40	1	48	27	24	6.40

Table 7 shows the results obtained with oleic acid and diglycol oleate. Contrary to the results obtained with lauric and capric acids, oleic acid had an immediately impairing effect on the clotting properties of raw skim milk, and temperature conditioning at 35° C. or 40° C. prior to making the curd tension test on the aged (at low temperature) sample was not nearly so effective in restoring the normal clotting properties. Similar results followed the addition of the oleic acid ester. It is noted that aging at low temperature caused a further impairment in clotting properties, analogous

to the lauric acid and capric acid additions. The increase in acidity obtained on aging the raw skim milk containing the added oleic acid ester was considerably less than for diglycol laurate. Whether this is due to differences in solubility and ionization of the fat acids themselves, or to differences in degree of hydrolysis of the esters was not investigated.

Another peculiarity of the oleic acid milks not brought out in the table was the appearance of a gelatinous precipitate in the sample held for sometime at 35° C. The material showed evidences of undergoing syneresis. There was no odor of lactic acid fermentation. That the phenomenon has some, as yet unexplained, significance is indicated by the fact that it also occurred in another experiment when using a much less pure sample of oleic acid, the details of which are not included in this paper.

5. *Effect of lipolysis on the clotting properties of whole milk.* An experimental study of this question is reported in table 8. In this study samples of fresh raw whole milk and separator skim milk from it, and aliquots of the same whole and skim milks which had been heated to 55–56° C.[8] for 30 minutes, were shaken vigorously for two hours in glass bottles in a shaking machine at 37° C. This should have induced lipolysis in the unheated samples, according to the results reported by Krukovsky and Sharp (3). The decline in curd tension (by the Hill knives at 35° C. without $CaCl_2$) on storage at low temperature which occurred only in the raw whole milk and the decline in pH, which was accompanied by development of butyric acid odor, support the conclusion that lipolysis of milk fat is detrimental to the clotting properties. The lipolysis was evidently too mild and too little of the fat acids especially detrimental to normal clotting liberated to duplicate the experiences with free capric, lauric and oleic acids. A com-

TABLE 8

Effect of induced lipolysis of whole milk on curd tension and pH

Sample no.	Description of sample	Curd tension		pH	
		Fresh*	After 24 hrs. aging†	Fresh	24 hr.
		gm.	*gm.*		
1	Raw whole, unagitated	54	53	6.63	6.64
2	Raw whole, agitated	32	22	6.47	6.42
3	Heated whole, unagitated	45	42	6.65	6.65
4	Heated whole, agitated	45	39	6.63	6.65
5	Raw skim, unagitated	73	63	6.62	6.61
6	Raw skim, agitated	73	69	6.62	6.61
7	Heated skim, unagitated	65	60	6.62	6.62
8	Heated skim, agitated	63	57	6.61	6.62

* These were tested immediately after agitation.
† Aged at 2° C.

[8] This temperature is stated by Sharp and de Tomasi (8) and by Mattick and Kay (7) to inactivate the lipase in cow's milk.

parison of samples 1 and 3, 5 and 7, 6 and 8, shows that the heat treatment itself had considerable detrimental effect on the strength of the rennet curd.

6. *Evidence that churning of natural cream may fail to induce lipolysis which affects curd tension of buttermilk.* Two experiments were conducted which bear on the question whether the normal churning of cream in butter making increases the lipolytic activity. If this is a normal occurrence it could account for the low curd tension of raw sweet cream buttermilk. The possibility that the agitation of churning may greatly increase lipolytic action was expressed by Krukovsky and Sharp (2). The evidence presented later (3) in support of this did not show any experiments at temperatures normally employed for manufacture of butter.

The experiments of Herrington and Krukovsky (4) seem to have a more direct bearing on the question; indeed, these authors conclude that a greater increase in fat acidity which was found to follow a longer storage of milks cooled at different rates was largely due to the agitation of churning employed to recover the fat for acidity determination, but this conclusion seems to have the fallacy that the only variable was the initial storage time, all samples being subjected to the same churning procedure and no evidence being given that the longer storage made it necessary to employ a longer churning time.

Since this question may be regarded as still open and in view of the evidence already presented that lipolysis can adversely affect curd tension under certain conditions it seemed important to obtain further experimental evidence on the possibility of a lipolysis in cream, induced by churning, causing the low curd tension of natural buttermilk.

TABLE 9

Curd tension and pH of buttermilks from churned creams prepared from reconstituted milks agitated at low temperatures

Sample no.	Description of whole milk giving creams from which buttermilks originated	Curd tension		pH	
		After 3 hr.	After 24 hr.	After 3 hr.	After 24 hr.
		gm.	*gm.*		
1	Raw skim + raw cream	55	56	6.50	6.42
2	Pasteurized skim + raw cream	42	39	6.55	6.55
3	Raw skim + pasteurized cream	49	48	6.49	6.42
4	Pasteurized skim + pasteurized cream	40	39	6.58	6.57

In the first experiment, shown in table 9, a lot of fresh raw milk from the station herd was separated immediately at 37° C. and both the cream and skim milk divided into two portions. One portion of each was heated at 65° C. for 30 minutes and the four portions then recombined to give the reconstituted milks, containing 3–4 per cent fat, described in that table. All were then agitated for one hour at low temperature (4–5° C.), reseparated,

at 37° C. and the cream portions aged for 16 hours at low temperature (4–5° C.). The creams were then warmed to 10–12° C., churned in glass Dazey churns and the curd tension of the buttermilks determined at once, at 35° C., using the Hill knives without $CaCl_2$, and again after 24 hours storage at low temperature. The results give some indication that the curd tension was affected by the heat treatment but there is no evidence from comparing samples 1 and 3, and 2 and 4, that lipolysis had been induced.

In the second experiment shown in table 10, it was planned to compare the effects of a normal churning temperature with those obtained at 25° C., when lipolysis would be more likely to be induced. There was a control lot of cream in which any lipase present was presumably inactivated by holding at 55–56° C. for 30 minutes. Additional controls consisted of samples of the original whole milk and skim milk which were also carried through the experiment. Curd tensions were determined by the Hill knives at 35° C., without $CaCl_2$.

TABLE 10

Effect on curd tension and pH of churning cream at normal churning temperature and at a temperature which should induce lipolysis

Description of sample	Churning temp.	Curd tension		pH	
		Fresh	After 24 hr.*	Fresh	24 hr.
	°C.	gm.	gm.		
Raw whole milk control	Not churned	65	61	6.56	6.57
Raw skim milk control	Not churned	74	68	6.58	6.59
Buttermilk from raw cream	25 – 10†	31	32	6.64	6.63
Buttermilk from raw cream	12	31	25	6.60	6.61
Buttermilk from heated cream	25 – 10†	21	19	6.65	6.65
Buttermilk from heated cream	12	24	18	6.63	6.67

* Storage temperature 2° C.

† The two temperatures given are the initial and final churning temperatures employed, as explained in the text.

In the actual carrying out of this experiment it was found very difficult to obtain butter at 25° C. None being evident after one hour of churning, the creams (both raw and heated) were cooled to 5° C. and churning continued at the lower temperature for an additional hour, again without result. These creams were then stored at the low temperature for 16 hours, after which they could be churned normally at 10° C. Undoubtedly the procedure which had to be followed in this experiment was more favorable for inducing lipolysis than that originally planned. The low curd tension found for the buttermilk samples is typical of raw sweet cream buttermilk but no evidence was found through change in pH or development of fat acids odors that lipolysis had occurred. However, the buttermilks from the heated creams showed the same effects of heat treatment evident in tables 8 and 9.

DISCUSSION

Krukovsky and Sharp (3) showed that hydrolysis of simple esters by milk is differentiated from "the true lipolysis of the natural fat" by the fact that the latter usually requires an "activation" by agitation at proper temperature. This would seem to offer a method of distinguished esterase from lipase in milk. However, the more recent work of these authors (9) showing that the "activation" mechanism seems to be merely that of altering the fat globule surface layer has no doubt changed their interpretation of the difference between the hydrolysis of simple esters and natural milk fat. Nevertheless, it is somewhat puzzling that the extent of agitation employed in dispersing diglycol laurate in raw skim milk influences directly the amount of hydrolysis subsequently obtained. This was shown in a previous paper (1). It seems impossible that violent agitation of skim milk to which diglycol laurate is added actually increases the dispersion of the ester because mere shaking of the ester with water is sufficient to produce a colloidal dispersion. Certainly the problem of natural versus artificial adsorption layers cannot be involved. Furthermore, violent agitation of skim milk would be expected to increase its oxygen content; this should decrease its lipolytic activity according to the recent paper by Krukovsky and Sharp (10).

The experiments with the various fat acids of butter fat point strongly to the conclusion that capric, lauric and oleic acids exert a specific effect in interfering with normal rennet clotting. This effect was manifested by the saturated fat acids only after aging the acid containing milk at reduced temperature. For oleic acid the effect was evident without aging, although increased by aging. Also the effect could be completely reversed by warming for a proper period of time at 40° C. or above, only in the case of milks containing capric or lauric acid. Considerable reversal occurred for these cases even at the normal curd tension determining temperature (35° C.) but the conditions employed in our experiments did not permit us to decide whether complete reversal would occur for the saturated fat acid milks at this temperature. The experiments showing the restorative effects of temperature treatment on lauric acid containing milk at first suggested that the melting point of the acid was related to the effect of heat treatment but this is completely ruled out by the capric acid results, the detrimental effects of which are manifested above its melting point.

It seems likely that the phenomenon is one of adsorption, the equilibrium of which is closely related to the temperature. The more pronounced immediate effect of oleic acid and the fact that this is not readily reversed, indeed perhaps never completely so, by temperature treatment alone, is probably related to the polar double bond of the acid, in addition to polar carboxyl group. The unsaturated bond evidently produced adsorption properties having a different temperature equilibrium. The phenomena

are being more closely investigated from this point of view, and the results will be reported later.

It is admitted that the experiments concerning the counter effect of $CaCl_2$ represent a rather superficial approach to the cause of this result. Although the experiments show clearly that allowing lauric acid first to come in contact with $CaCl_2$ in chemical equivalent amounts prevents the detrimental effect of the lauric acid, thus suggesting that the counter action of $CaCl_2$ is the result of a chemical reaction between it and lauric acid; nevertheless, insufficient information is available to permit a clear picture of the processes involved. Also, as stated previously, the possibility cannot be ignored that the reduction in pH caused by the $CaCl_2$ has a minor role in the reactions.

When the effects of the lauric acid were first discovered the possibility was at once presented that certain cases of poor clotting, particularly that of raw sweet cream buttermilk, might be the result of lipolysis of milk fat. The experiment reported with whole milk not only supports the conclusion that lipolysis may adversely affect the curd tension but is in line with the theory of Krukovsky and Sharp (3, 9) that lipolysis is greatly accelerated because of the "resurfacing" of fat globules brought about by their agitation in the liquid state. However, the churning experiments do not indicate that this is a normal occurrence during the manufacture of butter. In any case only a few of the fat acids which might be liberated will affect rennet clotting. Oleic acid, being by far the most dominant of these in butterfat, unquestionably plays the major role in interfering with the formation of a normal clot.

CONCLUSIONS

1. Raw skim milk and whey contain an esterase(s) which accelerate(s) the hydrolysis of diglycol laurate, diglycol oleate, and other esters at temperatures below 10° C. The enzyme(s) can be removed by ultra filtration and inactivated by heat.

2. The fat acids liberated from diglycol laurate and diglycol oleate in milk by enzyme activity or added directly to milk will, under proper conditions, seriously interfere with and even inhibit entirely the clotting of the milk by rennet. Capric acid is the only other common fat acid in milk fat which has this effect.

3. The adverse effects of the saturated fat acids are shown only after a period of aging at reduced temperature and addition of rennet at 35° C. (or lower), and may be completely reversed by heat treatment for one-half hour or more at 40° C. Some reversal occurs during several hours heating at 35° C. The adverse effects of oleic acid occur without aging and are not overcome completely by the same heat treatment.

4. The clot-preventing effects of lauric acid are not evident at 35° C. if $CaCl_2$ is added to the milk in the curd tension test. This is possibly due largely to a chemical reaction between the salt and the acid and (or) its

compounds, but may also be due indirectly in part to the lower pH of the $CaCl_2$ treated milk..

5. When lipolysis is induced in raw whole milk by agitation at proper temperature and subsequent holding at low temperature, some interference with normal rennet clotting results. Similar lipolytic effects are not necessarily induced in raw cream by churning, although the curd tension of the buttermilk will be low. The theory of protein denaturation, involving the fat globule protein, presented in an earlier paper (1) may be invoked to explain this result.

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VITAMIN D IN MILK—A REVIEW[1]

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INTRODUCTION

The introduction of vitamin D milk was based upon nutritional facts: (a) that an antirachitic factor is necessary for proper generation, growth and maintenance, (b) that this factor is lacking in dietary products normally used, including normal cow's milk, (c) that living standards precluded availability to humans of the natural agents by which the factor might be generated, (d) that milk contained the balance of a necessary nutritional triad, vitamin D, calcium and phosphorus, and (e) milk is generally available where there is need for the additional factor.

While these facts may be evident to the student who has reviewed the subject, less evident facts need some explication. The creation of a vitamin fortified milk simultaneously brought with it merchandising problems. Among these may be cited (a) the necessity of translation and transfer of knowledge of the biological laboratory and its precise and complicated terms into acceptable form for reading matter, radio and minds of the distributor salesmen and consumer (b) by inference there was tacit admission publicly of an inadequacy of cow's milk as a "perfect" food and (c) the change in equipment and processing methods, as well as prevailing regulations required a premium charge for the new vitamin-milk product.

Variation of Vitamin D in Milk. The preformed antirachitic quality of cow's milk is affected by secretory processes, feed quality and animal management. While the quantity occurring naturally in milk is far less than demanded by human dietary requirements, the variations and sources of the factor are of practical significance.

Range in Variations. The extreme spread of a number of reports of variations in the natural vitamin D content of milk is from 3.1 to 56.0 U.S.P. units per quart, a variation of some 18 fold. An average of the minimum and maximum levels cited in these studies is 19.3 and 31.7 U.S.P. units per quart, a variation of 1.6 fold. The amounts cited include variations due to the method of preparing the sample for assay, and the interpretation of the assay. Reference to factors affecting the variations in content of vitamin D in milk are of greater interest. This was excellently reviewed by Olson and Wallis (84).

Effect of Ration. While there have been a significant number of studies on the effect of several factors on the vitamin content of milk, the methods and results of most are insufficient to demonstrate the effects only of feed

[1] Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

quality. Wallis in carefully controlled experiments (86), depleted six Holstein cows of their reserves of vitamin D by feeding a deficient ration and keeping them from exposure to sunlight. This group of six cows was divided into three groups of two each, fed daily either 20 pounds of alfalfa hay containing 10,000 I.U. of vitamin D, 20 pounds of prairie hay containing 5000 I.U. of vitamin D or a ration containing as roughage beet pulp essentially devoid of vitamin D. The potency per quart of milk from the three groups at the beginning of the experiment was 5.4 I.U. After three months the cows consuming alfalfa secreted milk containing 13.0 I.U., those consuming prairie hay secreted milk containing 8.4 I.U., while the milk from the cows consuming beet pulp contained a quantity considered immeasurable. Only a small percentage (1–2 per cent) of the vitamin D consumed was secreted in the milk. It is interesting to note that when the vitamin intake of the cows is maintained, a reasonably uniform quantity per quart of milk during the lactation period may be expected (83). Luce, using a Jersey cow showed that the vitamin D potency of the milk of a cow protected from light was improved when the ration was changed from dry fodder to freshly cut meadow grass and clover. In studies by Krauss *et al.* (53), cows fed a high protein ration consisting of alfalfa with small amounts of corn silage secreted milk containing more vitamin D than cows fed a low protein ration consisting of timothy hay with liberal amounts of corn silage. It was concluded that these results were probably due to the greater intake of vitamin D from the alfalfa than from the timothy.

Effect of Breed. The total transfer of vitamin D from the normal feeds to milk is approximately the same for different breeds as shown by Wallis (83). When individual cows of the Holstein and Jersey breeds were fed 19,056 I.U. of vitamin D in 12 pounds of alfalfa hay daily, the percentage recovery in the milk was 0.47 and 0.51 respectively. The Holstein milk, however, contained about 10 I.U. and the Jersey 30 I.U. per quart, the fat of the Jersey milk being about 50 per cent more potent than the fat of the Holstein milk. A reciprocal decrease in vitamin D potency per gram of fat occurred as the percentage fat of the milk increased with progress of the lactation period. The milk flow of the Holstein cow was sufficiently large to make the transfer of vitamin D from the feed approximately the same for both animals. Under normal and parallel conditions of management, the milk of eight Guernsey cows was shown by Bechtel and Hoppert (5) to contain greater antirachitic value per quart than the milk of fourteen Holstein cows. Little difference in the potency of the fat from the two breeds was reported in these studies. Similar conclusions were made by Kon and Henry in England (47) who reported that the vitamin D of Guernsey and Shorthorn milks could be expressed by the ratio of fat percentages.

Effect of Lactation Period. As for other important nutritional factors, the vitamin D content of colostrum is greater than that of the milk from

the post-colostrum period. Henry and Kon, in a test of the churnings from a Guernsey cow in the fourth lactation found 1.22 units per gram in the milk of the first day, 0.56 in the milk of the next two and one half days, 0.36 in the milk of the following day interval, and 0.41 in the normal milk (48). This is found equivalent to 119, 55, 35, and 40 units per quart. Van Niekirk and Bleek reported even greater concentrations in the colostrum being computed at 236, 132 and 110 units per quart for three fractions, whereas two normal milk samples were found to be 17 and 24 units, respectively (49).

When fed a uniform amount of vitamin D, the vitamin D in the milk of one Jersey and one Holstein cow was found to be reasonably uniform over the entire lactation period (83).

Influence of Sunshine. That sunshine, which provides definite antirachitic aid to humans, properly exposed, affects the antirachitic value of the milk of cows, has been adequately demonstrated. In the second of two experiments by Luce (57, 58), a Jersey cow was housed for two periods in a dark stall and two periods in the open sunshine. Improved antirachitic quality of the milk was shown when the cow was exposed to sunshine whether a vitamin-poor dry ration or a green-feed ration was employed. Chick and Roscoe (13) Dutcher and Honeywell (16) have made observations confirming the beneficial effect of sunlight. Olson (64) raised two groups of four each of heifer calves, one kept in the sunlight, the other in a dark run-shed. Similar rations were given to both groups. The milk from one each of the heifers in their first lactation was fed to separate groups of pigs. Those pigs receiving milk from the cows housed continuously with access to sunlight showed greater weight gains and freedom from rickets than those receiving milk from the dark stall cows.

Campion and co-workers (11) showed that whether eight Shorthorn cows in groups of two each were fed a winter ration or a summer ration, the effects of exposure to sunshine were so marked as to lead to the conclusion that increases in potency in milk in the summer are due entirely to the sunshine. With a winter and summer ration respectively, fed to cows indoors, the milk contained 8.2 and 5.2 I.U. and when fed to cows outdoors, the milk contained 24.0 and 16.4 I.U. per quart, respectively. A number of investigators have correlated vitamin D potency of milk or milk fat with the prevalence of sunshine. The studies of Wilkinson (94) show excellent correlation between the vitamin D content of Scottish butter and the amount of sunshine.

Effects of Artificial Irradiation of Cows. Probably because of the known antirachitic effects of sunlight acting upon certain animals, fowl, and feeds, the use of artificial sources of radiation for the application of ultra violet radiation upon lactating animals has been investigated. Uniformity in results did not occur in these experiments. When used for feeding chickens, the milk of cows irradiated 15–30 minutes daily by use of a quartz-mercury

vapor lamp was found to have increased antirachitic potency (24, 25). The milk of cows irradiated by a quartz-mercury vapor lamp was reported to prevent rickets in infants normal at birth, but inadequate for the cure of rickets in rachitic cases (8). The antirachitic value of the milk of a lactating goat was increased decidedly by the use of mercury-vapor-arc radiations, although in this case ultimately the goat showed a negative calcium balance (71).

On the other hand, an extensive experiment has shown that application of ultra violet radiation from a quartz-mercury-vapor lamp to various quarters of lactating cows for four weeks was without significant effect upon the antirachitic potency of the milk (72). The variations observed in the course of the experiments were not as great as observed when the cows were differently managed according to seasonal factors.

Because calves have been shown to be protected from rickets with radiations from a quartz-mercury-vapor lamp (65) it is probable the response to the radiations by a milking cow is affected by such factors as the rate of production and adequacy of the ration. While exposure of cows, as well as other animals, to sunlight is a recommended practice, the economical advantage of artificial radiation for cows except in unusual cases, seems questionable.

Seasonal Effects. In view of the several factors which affect the normal vitamin D potency of milk, the occurrence of seasonal differences is not surprising, and such observations have been cited in several reports (84, 72, 74). Recently, Wilkinson (94) showed a sharp summer peak in vitamin D activity of Scottish and Danish butters. The highest, lowest and mean values in I.U. per gram were, for Scotland butter, June, 0.99; March, April and December, 0.08, mean 0.32; and for Denmark, July, 0.54, December, March, and November, 0.08, mean 0.23. Wallis and Olson (84) reported values for April, 0.23, July, 0.83 and November, 0.28 I.U. per gram. Bechtel and Hoppert (5) found the greatest values in July, August, and September and the lowest usually in February and that the potency may vary as much as 900 per cent over the seasons.

Vitamin D in Feeds. Not the least of nutritional problems is the adequacy of feeds for livestock. Since the vitamin D content of milk is, in part at least, affected by the vitamin content of the feeds consumed, and in view of the other physiological demands of the lactating cow, the provision of adequate amounts of this factor in feeds is no less important than for other factors. The method of curing and handling hays, and the selection of feeds for lactating cows has been shown to be important in making provisions for vitamin D requirements. Unfortified grain concentrates (65, 41) oils (7, 60) from corn, cottonseed, linseed and peanuts, roots and succulents as turnips, beets, cabbage, carrots, parsnips and potatoes (14) are very poor providers of the vitamin D factor.

The leafy portion of good quality green colored hay was found to be six times as potent as the stems, the concentration being 10.45 and 1.72 I.U. per gram, respectively. It is evident that overcuring and shattering of hay crops should be avoided. The use of a sun-cured leaf meal as a good antirachitic has been suggested (85). The quantities of vitamin D reported in roughage vary considerably and range from 500 U.S.P. units per pound for sun-cured alfalfa hay, 150 units in prairie hay, 27 units in New Zealand hay, and 40 units in moist corn silage (84, 15, 4). Oats straw has been found a reasonably good natural source of the factor (3). There is little evidence that vitamin D as such occurs in live plant tissues (6). But the degree of insolation of hay crops is an important factor in enriching them with vitamin D. Clover, timothy, and alfalfa hay when cured in sunlight contain significantly greater antirachitic value than when cured in the dark, or when artificially dried (70, 67, 68, 3). Besides shattering, excessive weathering of clover hay exposed to dew and rain has been shown to be destructive of the antirachitic value (70).

Dairy Cattle Requirements of Vitamin D. There is increasingly available evidence of the necessity of having adequate amounts of vitamin D in the rations of dairy cattle and calves. In calves, inhibition of growth, decline in blood phosphorus and serum calcium, and manifestations of rachitic symptoms such as stiffness of the animals, swollen joints and abnormal posture of knees and pasterns and rachitic tetany follow utilization of rations low in the antirachitic factor (65). Rachitic symptoms in calves have been produced by use of experimental vitamin-deficient rations. The development of rickets in calves fed what might be considered "normal" rations has been observed in both practical and experimental operations. The use of winter and summer whole milk with a basal ration, or of milk alone may not be a preventative of rickets (59, 12, 38). It has been shown in these studies that the development of active rickets or its symptoms in calves may be prevented by the use of adequate amounts of properly cured good quality hay, exposure to sunshine, and the use of vitamin D supplements. If unsatisfactory rations and inadequate sunshine in the winter are a common regime, then the use of vitamin D supplements for calves may be considered proper. The studies of Bechdel and associates indicate that the daily requirement of vitamin D of calves is between 250–450 I.U. per hundred pounds of body weight of which a minimum supplementary requirement was estimated to be 100 to 300 units.

The significance of adequate provision of antirachitic factor for calves as they mature into lactating animals is not at once evident, but presumably is so. Olson (64) reported that two cows confined from sunshine for three lactation periods suffered a marked fall in milk production by the third lactation, although sun-cured alfalfa was fed. In comparison with groups of calves matured in a similar manner except with exposure to direct sun-

shine, no difference in blood calcium or inorganic phosphorus, or reproduction of the calves was noted. The dependency of offspring on the maternal supply of vitamin D has been dramatically shown by the experiments of Wallis (82) wherein cows on rachitic rations dropped manifestly rachitic and deformed calves. The very frequent negative calcium and phosphorus balances observed in heavily lactating cows seem not to be corrected completely by administrations of calcium and phosphorus and vitamin D. According to Rupel and co-authors, "apparently the milk secretory function is so dominating that the effect of vitamin D additions through food or light is submerged to the point where its effect is not easily measurable. The feeding of vitamin D in yeast to milking cows at the height of their lactation did not improve the mineral balances although vitamin D was absorbed and the milk enriched in vitamin D" (65). Experiments by Wallis showed that the calcium and phosphorus of blood plasma of lactating cows kept on vitamin D deficient rations declined to one-half and one-fifth, respectively, the normal plasma levels. The cows manifested typical rachitic symptoms. When fed vitamin D the mineral losses of the animals were changed to significant retentions. It is probable the extent of response to administrations of vitamin D in these experiments (65, 82) was dependent upon the various factors such as condition of the animal, volume of milk secreted, and so on. The possibility of deficiency of vitamin D in the ration as a factor of the onset of milk fever has been frequently mentioned. A seasonal cycle of greater frequency of milk fever in January, February, March, and April when solar radiation is poorest, and the effects of antirachitically poor rations fed over a period of time is believed probable since supplementing of the ration for some time before parturition has been found beneficial (32, 69).

These studies indicate that over the long period of time, inadequate supplies of vitamin D in the ration of cows, and calves is reflected in the antirachitic quality of the milk secreted.

FORMS OF VITAMIN D OCCURRING IN MILK

There are several different forms of vitamin D, and it is probable that at least three forms occur in the different types of commercial vitamin D milk available. It is also probable that at least two forms occur in milk as elaborated by the cow. These two forms are activated 7-dehydrocholesterol or vitamin D_3, and activated ergosterol, frequently called calciferol, or vitamin D_2. Two additional forms, activated 22-dihydrocalciferol, or vitamin D_4, and 7-dehydro-sitosterol or vitamin D_5 also might be present.

Cholesterol is a normal constituent of body tissue, the blood, and milk. When acted upon by ultra-violet radiation, it is transformed into vitamin D_3 (7-dehydrocholesterol). The concentration of cholesterol in milk fat is about 0.3 per cent, and in skin fat about 19 per cent (18, 1). It is probable

the activated cholesterol formed by the solar radiation acting upon epidermis of cows exposed to sunlight is transported to the mammary gland and there secreted, in part at least, in the milk. Aside from the experiments showing the effects of sunlight (and artificial radiation) on cows and the vitamin D potency of their milk, and the correlations between seasonal trends in the potency of milk and milk fat and solar radiation, there is little direct (immediate) evidence of the transfer of this sterol activated *in vivo* to the milk. Nevertheless, in view of the ubiquity of the sterol in animal tissue, the knowledge that cholesterol is provitamin D (81, 10), and that solar radiation is of definite antirachitic value to animal life, there seems ample reason for assumption that the factor is thus transported to milk.

Ergosterol, a vegetable sterol, is believed a normal constituent of plant tissue, and is found in yeasts and molds. This substance, acted upon by ultra-violet radiation of from 2300–3100 Å, is transformed in a series of intermediate steps and products into calciferol, or vitamin D_2. Because of its vegetable origin, it is presumed that the principal sterol of forages acted upon by solar radiation when sun cured is ergosterol. Because, as will be discussed, the calciferol of irradiated yeast when fed to cows is passed through the blood stream to milk, it is also presumable that this vitamin as well as the forms D_4 and D_5 of forage crops is similarly translated to the secreted milk. It is interesting to note that for the cow, the unirradiated ergosterol evidently is not transmitted from the feeds to the milk (56). It has been suggested by Bills (8) that possibly the provitamins of vitamins D_4 and D_5 occur in the phytosterols of the plant kingdom. If so, the vitamins may be present in sun cured forage and appear subsequently in the milk of cows. The constitutional form and characteristics of the various forms of vitamin D have been adequately reviewed by Bills.

INCREASE IN VITAMIN D IN MILK BY BIOLOGICAL MEANS

It has long been known that when substances rich in vitamin D are fed to lactating animals, a definite increase in the antirachitic value for the milk can be obtained. Among the substances rich in vitamin D fed to cows and which have caused an increase in the factor in the milk are cod liver oil, irradiated yeast, irradiated ergosterol, and irradiated molds. Of these the feeding of irradiated yeast has been the most frequently studied, and proven the most practical.

Yeast, of which there are many strains, contains several sterols, among which may be included zymosterol, cerevisterol, and 22-dihydroergosterol. The amount of the activatable provitamin D ergosterol in yeast is dependent upon the strain, but in some forms may approximate as much as 2–3 per cent. A suspension of yeast, removed from the spent wort, is exposed to ultra-violet radiation, and the provitamin ergosterol is converted into the active form of vitamin D_2. When fed to cows, the vitamin D_2 is absorbed

in the blood stream and transferred *via* mammary gland to the milk. The conditions under which the yeast is fed affect the rate and completeness of transfer of the factor to the milk.

When a cow is fed irradiated yeast (108,500 U.S.P. units) the absorption of vitamin D from the intestine, as measured in the blood, begins within one to two hours and reaches its maximum by the second hour (55). Calculations indicate that practically 100 per cent of the vitamin D fed appears in the blood stream. While this transfer seems efficient, the ultimate transfer of the factor to the milk is relatively inefficient and approximates some 2 to 3 per cent (36). The major portion of the vitamin D absorbed seems to be destroyed in the body, for only a portion (25 per cent) of the vitamin D ingested is eliminated by way of the intestine. It is not believed that the vitamin D (unaccounted for) is stored in unusual amounts in the tissues, since deposits of the factor have not been located, although it may be retained over a period of weeks in various organs of some species (37). The secretion of vitamin D in the milk of a cow approximately parallels the level of vitamin D in the blood. The concentration of the factor in the blood plasma therefore seems to govern the concentration of the factor in the milk (55). The higher concentration of vitamin D in the blood after ingestion of the antirachitic feed explains the greater efficiency of transfer of the factor from the feed to the milk by high producing cows. Higher producing cows secrete a greater percentage of their total production in the first half the interval between milkings than do low producing cows. When fed the same amount of vitamin D in the ration, a greater amount of vitamin D is recovered in the milk of the high producing cow. Reduced to practice, when a desired level of vitamin D is desired per unit volume of milk, relatively less irradiated yeast need be fed cows that secrete a greater volume of milk (22). It is interesting to note that while decreased feeding of irradiated yeast caused a proportionately lower content of the factor in the milk, multiple increases of yeast in the ration were not accompanied by corresponding increases of vitamin D in the milk. The cow appears able to utilize the vitamin D effectively only within a certain range. The vitamin D recovered in milk from cows fed irradiated yeast is concentrated essentially in the fat fraction of the milk. In studies where irradiated yeast was fed cows, it was concluded that the lower the total production of butterfat, the greater the concentration of the vitamin in the fat, and further, the greater the daily production of the milk by the animal the greater will be the total number of vitamin D units excreted in the milk per day, although the number of D units per unit volume of milk may be less (36). On the other hand, it was observed by Wallis (87) that the vitamin D concentration in the blood of Jersey and Holstein cows fed daily a normal ration containing 7500 I.U., was the same, as were the concentrations in the butterfat. Since the Jersey breed produced more butterfat, the total amount of vitamin

D secreted by the breed also was high. The differences evident in the two observations are probably due in part to the use of a fortified ration on the one hand, and a normal ration on the other. The feeding of moderate supplements of vitamin D in the form of irradiated yeast to cows in amounts (60,000 I.U.) adequate for a milk product of the desired antirachitic level (400 U.S.P. units) has no significant effect upon the milk or serum phosphorus, calcium or milk ash. The feeding of supplemental quantities of yeast to cows over a period of years has not been found harmful to cows and on the basis of known physiological requirements is believed helpful.

The effectiveness of different sources of vitamin D fed cows for increasing the vitamin activity of the secreted milk is by no means the same. The vitamin D in irradiated yeast (irradiated ergosterol or calciferol) has been reported from two to three times more effective than the vitamin D in the form of viosterol (irradiated ergosterol or calciferol dissolved in corn oil) (35). On the other hand, the studies by Russell *et al.* show similar efficiencies for the two products when fed at moderate levels (60,000 units per day) and slightly better efficiencies for the yeast when the two products are fed at levels of 180,000 units per day (66). By comparison the feeding of vitamin D in cod liver oil extract (Vitex) also appeared less efficacious (51, 52). The feeding of cod liver oil as such has been shown to have a depressing action on the secretion of milk fat and is poorly if at all adsorbed from the intestinal tract of the cow (28, 23, 27). Cocoa shell, an important by-product of chocolate manufacture and a feed for cattle, acquires, during the exposure to sunshine and subsequent fermentation by yeasts, considerable amounts of vitamin D, approximately one-quarter that of average cod liver oil (46, 43). The cocoa shell contains 28–35 I.U. per gram and the cocoa shell fat about 300 I.U. per gram, unusual values for a vegetable product. When fed in levels of 32,000 I.U. daily (2 pounds cocoa-shell per cow) an increase in potency from 8.36 to 19.4 I.U. per quart was noted (computed for 4 per cent milk).

INCREASE OF VITAMIN D IN MILK BY ADDITION OF CONCENTRATES

In addition to the use of metabolic processes, the vitamin D content of milk may be increased by direct addition of the agent. Several forms of vitamin D are employed for this purpose. These are of different origin or are prepared by different methods. Of the forms used commercially the vitamin D is dispersed in a conveying agent, either of a readily miscible or dispersible oil, or in a homogeneous fluid mixture of milk constituents. These products, referred to frequently as concentrates of vitamin D, are measured in computed quantities and added to the milk to be fortified prior to its pasteurization. The amount of vitamin D in the concentrates varies for different commercial products, but approximates 4000 U.S.P. units per cubic milliliter. When used at the rate of 1.0 cubic milliliter per 10

quarts of milk, an added potency of 400 U.S.P. units per quart of milk is obtained. One ml. of concentrate added to 10 quarts of milk is equivalent to 0.0105 per cent of the original volume of milk.

Three forms of vitamin D concentrate are available (93):

(a) Vitamin D prepared from extracts of fish liver oil, and dispersed in milk constituents. The vitamin from this source is believed to consist chiefly of the cholesterol or D_3 type (8).

(b) Vitamin D (calciferol) prepared by the activation of ergosterol, either by ultra-violet, or electron radiation, and dispersed in milk constituents. A commercial source of the ergosterol is yeast. This form of vitamin D is the calciferol or D_2 type. The use of ultra violet activated – 7-dehydrocholesterol or D_3 dispersed in milk constituents in the same manner as for calciferol is entirely feasible (89).

(c) Vitamin D prepared by activation of ergosterol with ultra-violet radiation as in (b) but dissolved in an oil carrier such as sesame oil plus an emulsifying agent.

The concentrates of the forms (a) and (b) dispersed in milk constituents may consist of the vitamin dispersed in evaporated milk (17), whole milk, or products of intermediate or similar composition, or of the carrying oil dispersed in a casein solution. The concentrates of these forms are readily dispersible in milk, and require no preparation for use save removal from the container. The concentrates, consisting of vitamin D in milk constituents, are generally preserved in cans by heat sterilization. The concentrates of vitamin D in oil are biologically non-perishable, but are preferably handled with use of refrigeration.

While more frequently associated with fluid whole milk, the concentrates are used in other fluid dairy products such as chocolate and buttermilk, in evaporated milk, and ice cream, and in food products such as bakery goods.

Little experimental research has been reported on the influence of the concentrate products of vitamin D on the various properties of milk. The quantities necessary for adequate fortification of the milk are so small that effects on flavor are undiscernible, and secondary reactions have not been cited. The flavor of most canned concentrate products is quite bland. They possess perhaps a slightly cooked flavor developed in the process of sterilization. The concentrates consisting of solutions in oils possess the characteristics of the oil employed. Vitamin C in milk was found uninfluenced by the addition of a vitamin D concentrate (95).

A significant number of patents are extant on carriers, and methods of dispersion of vitamin D. Few of these are feasible for application in the dairy industry.

INCREASE OF VITAMIN D IN MILK BY IRRADIATION

The process of enhancing the vitamin D content of milk has been discussed recently in a review bulletin (92).

By the process of irradiation, milk in thin films is exposed for short periods to radiation of suitable quality and intensity so as to acquire a desired degree of antirachitic potency. Commercially, potency levels of 135 and 400 U.S.P. units per quart are attained.

The increase in vitamin D is the result of the action of radiation of wave lengths from 2300 to 3100 Å on the activatable substances in milk, believed to be principally 7-dehydrocholesterol. The sources of radiation employed are either carbon or quartz-mercury-vapor arcs. The films of milk are exposed for periods of approximately one to four seconds, respectively, at flow capacities of 350 to 1,000 pounds per linear foot per hour. The thickness of the films under these conditions of operation is approximately .04 to .06 centimeter. There are a number of factors which affect the response of milk to radiation that may be summarized as follows: composition of the milk—concentration of activatable substances and opacity of the milk to radiation; characteristics of the film—thickness and velocity; characteristics of the radiation—intensity and composition; mechanical principles—film capacity, film dimensions, distance of the source of radiation, and incidence of the radiation.

The variable mechanical factors have been controlled by employment of fixed mechanical parts and automatically operating control mechanisms. Because of the investment in equipment necessary, the economical production of irradiated milk is limited to operations capable of handling from 2,500 to 15,000 pounds or greater per hour.

The radiation, properly applied, is known to have no significant effect upon the other vitamin factors in milk. Improperly or unduly applied, the radiation may cause the development of the "sunshine" flavor, which develops also by action of solar radiation acting upon the proteins of milk. Either fluid milk or evaporated milk is processed by the irradiation method. While the antirachitic potency of dried milk may be increased by irradiation, the process generally employed consists of irradiation of fluid milk prior to its dehydration (79).

Since the publication of the review on milk irradiation (92), several developments have been announced. Of primary interest is the trend toward employment of sources of radiation having greater emission of energy in the effective ultra violet spectrum. Quartz mercury vapor arcs have been developed to consume greater inputs of energy, with consequent greater output of ultra violet radiation. Carbon electrodes too have been developed to handle greater inputs of power with greater emission of ultra violet radiation. These developments permit attainment of two objectives; greater flow capacities of milk for given operating units, and/or increased antirachitic potency in the milk. Whereas the quartz mercury-vapor arcs formerly used operated with wattage inputs of 1250, the more recent types may operate with inputs of 2700 watts. The recently introduced W carbon

electrode is reported to have approximately 50 per cent greater ultra violet emission than does the U type carbon, and may be operated at greater power inputs.

The extent of activation of milk in the various depth planes of vertical flowing films having different capacities and exposed to normally used sources of radiation has recently been re-examined (2, 63). The techniques employed in the most recent of these studies consisted in activation of milk, and of ergosterol, and the decomposition of potassium persulfate by radiation transmitted through the flowing films, and the microtome fractionation into two or three parts, of irradiated flowing films having different volume capacities. Surface halves of average films possess, generally, but slightly better than half the potency acquired by the total film. The results of application of radiation to quiescent films following intervals of turbulence appear not to differ significantly from the results obtained when films were irradiated while either continually quiescent or turbulent (94).

In a series of experiments, the source and character of the flavor in milk exposed either to solar or ultra violet radiation has been elucidated. The flavor of milk exposed sufficiently to radiation transmitted through glass, or exposed to ultra violet radiation for a sufficient period is known to originate with the protein fraction, principally the casein and albumin, of milk. The exposure of relatively pure preparations of the amino acids to radiation has led to the conclusion that cystine, tryptophane, methionine and histidine are probably the important sources of the typical "sunshine" or "activated" flavor (90, 91, 19, 20). A procedure for isolation of the fraction of milk responsible for activated flavor has been developed (21). Milk exposed to radiation for prolonged periods undergoes an increase in sulfhydryls, the concentration of which may be determined in the distillates by a colorimetric procedure.

STABILITY OF VITAMIN D IN DAIRY PRODUCTS

The vitamin D of natural or fortified food products is quite stable. The vitamin D of milk is stable to the ordinary thermal applications of pasteurization (88, 79) at 62.8° C. for 30 minutes or sterilization at 115.6° C. for 15 minutes (88), as employed for evaporated milks. A major portion of the vitamin D concentrates used for the fortification of market milk are canned and sterilized products. Vitamin D_2 (calciferol) is unstable at greater temperatures such as 232.2 to 260° C., moist state, for periods of five minutes. The naturally occurring vitamin D, and induced vitamin D, are stable during long periods of storage in products such as butter, and evaporated milk (88). The vitamin D induced in milk at experimentally high levels by ultra violet radiation (several fold more than the level attained by commercial processes) has been reported as unstable even for relatively short periods of storage (62). Vitamin D in dry yeast, stored at room temperature in a

stoppered jar, which was opened and the contents mixed at intervals, was found stable for over a period of six years (78). The vitamin D in eleven evaporated milk samples stored at normal stock room temperatures (40–110° F.) for three years was found stable in all samples after one year, and in all but a few after two and three years storage (54).

MEASUREMENT OF VITAMIN D IN MILK AND PUBLIC HEALTH CONTROL OF THE PROCESSES

Up to the present time, no reliable and accepted rapid technique has been developed for the estimation of vitamin D in milk. The official method (76) consists of the use of animal bioassay methods, requiring some three weeks for the preparation of animals, and a period of 10 days' feeding of the substances being examined. Because of the expense of conducting such tests, and the necessity for employing trained personnel, only a limited number of laboratories provide bioassay service for vitamin D measurement of foods for public service. For example, the only city-maintained vitamin D testing laboratory is that of New York City, and the only officially designated state laboratories for the testing of vitamin D in foods are those of Connecticut and Virginia. Elsewhere, the service is provided for food regulatory divisions of city health departments by arrangement with laboratories, such as at Michigan State College, Ohio Agricultural Experiment Station, Rutgers, and at private laboratories having interest in the various vitamin D processes or products.

Because of the expense of conducting a bioassay for vitamin D in a food product, the tests made upon a product of a given plant are infrequent, and may vary from periods of monthly, to quarterly, or semi-annually, depending upon the requirements of the management, or of the health regulations. Under these circumstances, regulatory officials rely principally upon the significance of clauses pertaining to labeling, unannounced sampling, and other sampling techniques. When the vitamin D milk is produced by the "metabolism method" by the feeding of irradiated yeast to cows, the yeast purchases and utilization records offer a means of checking the probable potency of the milk. Manufacturers of the yeast also provide to officials certifications of the potency of the yeast being fed to the cows. In a manner similar, producers of concentrate products of vitamin D provide certifications of the potency of the concentrates employed by milk distributors. In addition to requiring records of the use of vitamin D concentrates in milk, and the milk produced, in at least one city the addition of the vitamin D concentrate to the milk is performed by a designated agent of the health department. When the process of irradiation is employed, a recording chart indicating both uniformity of power input and milk flow are available for purposes of record.

LABELLING REGULATIONS

In the food regulations of some states, the legal definition of milk is so rigid as to prohibit the use alone of the term "milk" with any product such as milk to which has been added an adequate amount of vitamin D. Further, some regulations, by their verbal structure, prohibit the addition of anything foreign to milk, and others, properly interpreted, prohibit the addition of anything to milk. Many state regulations defining the term milk, and qualifying its use, were promulgated at a time when practices of watering, skimming and chemically preserving were more common than at present. Many regulatory officials, therefore, have not chosen to interpret the regulatory definition of the term "milk" to exclude the addition of a nutritional adjunct, since the fortification of milk with this nutritional factor (D) is in the interests of, rather than contrary to, the status of public health.

This problem has been satisfactorily handled in most of the regulatory districts by permitting the labelling of the product as "Vitamin D Milk," or "Vitamin D Added," to distinguish it from milk not containing added vitamin D. Additional requirements of labelling in some regulations is citation of the amount of vitamin D added, and the source of, or process employed in, augmenting the vitamin D in the milk.

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RULES FOR THE HERD IMPROVEMENT REGISTRY TEST*

RECOMMENDED BY THE

AMERICAN DAIRY SCIENCE ASSOCIATION

The following uniform rules have been adopted by the American Dairy Science Association for the conduct of Herd Improvement Registry Tests throughout the United States:

1. RULES MUST BE FOLLOWED.—The supervisor is not at liberty to decide as to which stipulations contained herein are essential and which are not, but is required to observe these directions in all details.

2. RELATIONSHIP OF SUPERVISORS.—The supervisor shall bear in mind at all times that he is the representative of the State College or Experiment Station and is not working for nor taking directions from the breeder, and his duty is to see that the test is honestly made and accurately reported.

3. ENTERING HERDS ON TEST.—Owners of registered cattle of any dairy breed may make application at any time to the national breed association concerned to enter their herds on test using the forms furnished by the breed association. The application should be made at least 2 weeks in advance of starting so that the breed association will have time to arrange for test supervision to apply to the first month of the herd test.

The herd test may be started at the first of any calendar month.

4. IDENTIFICATION OF COWS ON TEST.—The supervisor shall study the registration papers or official diagrams of each cow carefully and be sure that each cow he is testing is the animal for which the registration papers were issued. Because of the difficulty of identification, each cow not having a distinctive marking must bear a tattoo mark in the ear. This mark must be recorded on the official report or the test is worthless. Unidentified cows are not allowed to be placed on test, but whenever the registration papers are not at hand, a sketch must be made of the color markings and a statement made on the report as to why it was necessary to enclose the sketch. This is also necessary when the color markings do not correspond with the registration papers.

5. CONDUCT OF TEST.—Twelve consecutive official test periods of 24 hours, approximately one month apart, without preliminary milkings, shall be required during the testing year, retests and surprise tests not to be included. Credits for milk shall begin with the fourth day after calving but no butterfat test shall be made before the seventh day. The day on which the calf is dropped is counted as the first day.

6. SUPERVISION AND INSPECTION.—The conduct of the Herd Improvement Registry Test shall be in charge of the Superintendents of Official Testing in the several states. In the appointment of all supervisors and inspectors,

* Includes all revisions to December 31, 1940.

the rules for the official test covering such procedure shall apply, and all supervisors shall be appointed and employed in the usual manner.

7. COWS TO BE TESTED.—All registered cows in a herd that have ever come into milk shall be tested and remain on test as long as they are in the herd except as specified in Rule 8.

8. COWS TO BE OMITTED FROM TEST.—The following exceptions from testing may be made:

a. Cows 12 years old or over with A.R., R.O.M., R.O.P., or H.I.R. records.

b. Cows used as nurse cows throughout the lactation period having one or more A.R., R.O.M., R.O.P., or H.I.R. record meeting A.R., R.O.M., or R.O.P. requirements.

c. Cows whose registration certificates are surrendered for cancellation before the 11th month of the Herd Test Year.

9. REQUIREMENTS OF MILK WEIGHTS.—The requirement of daily milk weights is optional.

10. NUMBER OF COWS MILKED AT ONE TIME.—Not more than two cows may be milked at one time, except where a combine milker is used and then not more than four units of one section may be operated by one attendant only.

11. TIMES DAILY COWS MAY BE MILKED.—Not more than three milkings per day may be permitted.

12. NUMBER OF COWS WHICH MAY BE SUPERVISED PER DAY.—Thirty cows, or if individual samples are taken, a maximum of 60 milkings may be supervised by the tester.

13. SAMPLES TO BE TESTED.—Composite samples but not in duplicate. Individual samples may be permitted. The preliminary milking where required shall be weighed, sampled, and tested for butterfat in the usual manner. The preliminary milking shall be considered as one of the regular milkings supervised daily.

14. RETESTS. (OBLIGATORY REQUIREMENTS.)—Automatic retests are to be required when 60 days after freshening cows exceed the following daily butterfat production:

	Lbs. fat in 1 day
Mature cows	3.0
Senior 4 years old	2.9
Junior 4 years old	2.8
Senior 3 years old	2.6
Junior 3 years old	2.4
Senior 2 years old	2.2
Junior 2 years old	2.0

All retests are to be made at the owner's expense.

15. SURPRISE TESTS.—One or more surprise tests with a preliminary milking may be made by a different supervisor during the testing year. Surprise tests shall be ordered at the discretion of the breed association superin-

tendent of advanced registry and it shall be left to his discretion as to whether the surprise test be in addition to or supplant the regular test.

The first surprise test is to be at the owner's expense, others at the breed association's expense.

16. OWNER'S REQUESTED RETESTS.—The herd owner may request a retest by notifying the state superintendent of official testing within 72 hours of the close of the original test. All cows in the herd must be included in the test and at the expense of the owner.

17. REPORT BLANKS.—The American Dairy Science Association Herd Test Report Blanks are to be used to report the tests.

18. YEARLY HERD AVERAGE.—In calculating yearly herd averages the production for the herd test year of all registered cows that have ever freshened except for such cows as are omitted from test under item 8, shall be included in the herd average.

The "cow year" method of figuring herd averages shall be employed.

19. COWS WHICH ABORT WHILE ON TEST.—If a cow on Herd Improvement Registry Test aborts while in milk, and the gestation is 152 days or longer, her current record shall end and a new lactation shall begin, but if the abortion shall occur at less than 152 days the test is to be continued to complete the 305 day or 365 day test period.

20. DISHONEST OR FRAUDULENT PRACTICE.—

a. If the state superintendent of testing is satisfied that fraudulent or dishonest practices have been used in making of herd test records of registered cows, he shall report same to the breed association which may reject or cancel such records.

b. The feeding or injection of thyroid extract or thyroxine to cows on Herd Improvement Registry Test is prohibited and evidence of its use shall be cause for cancellation of such records.

21. HEALTH OF SUPERVISORS.—Supervisors conducting inspection on herds entered on Herd Improvement Registry Test shall be required to possess health certificates conforming to the same rules regarding health as required of milkers and other employees in dairies producing milk under regulations of the Standard Milk Ordinance, United States Public Health Service.

22. MATTERS NOT COVERED BY RULES.—Details of supervision which these rules do not specifically cover shall be administered by the state superintendent of official testing.

It is recommended that the Uniform Herd Test Rules of the American Dairy Science Association be used as a basis of enforcement where controversies arise.

23. REVISION OF RULES.—Any revision of the rules shall be made by a joint committee of the breed associations and the American Dairy Science Association. The chairman of the Breeds' Relations Committee shall be ex-officio chairman.

RULES FOR THE SUPERVISION OF OFFICIAL TESTS*

RECOMMENDED BY THE

AMERICAN DAIRY SCIENCE ASSOCIATION

The following uniform rules have been adopted by the American Dairy Science Association for the conduct of Official Tests throughout the United States:

1. RULES MUST BE FOLLOWED.—The supervisor is not at liberty to decide as to which stipulations contained herein are essential and which are not, but is required to observe these directions in all details.

2. RELATIONSHIP OF SUPERVISORS.—The supervisor shall bear in mind at all times that he is the representative of the State College or Experiment Station and the Breed Association and is not working for nor taking directions from the breeder, and his duty is to see that the test is honestly made and accurately reported.

3. IDENTIFICATION OF ANIMAL.—The supervisor shall study the registration papers or official diagrams of each cow carefully and be sure that the cow he is testing is the animal to which the registration papers belong. Because of the difficulty of identification, each cow not having a distinctive marking must bear a tattoo mark in the ear. This mark must be recorded on the official report or the test is worthless. Unidentified cows are allowed to be placed on test, but whenever the registration papers are not at hand, a sketch must be made of the color markings and a statement made on the report as to why it was necessary to enclose the sketch. This is also necessary when the color markings do not correspond with the registration papers.

4. POSSESSOR CONSIDERED OWNER.—For all purposes of official testing work, the possessor is considered the owner and is required to treat the animal as such, presenting all necessary data, the same as for his own.

5. NUMBER OF MILKINGS TO BE SUPERVISED AT ONE TIME.—No supervisor is allowed to supervise more than thirty-six (36) milkings per day, regardless of the method of milking—machine or hand.

6. PRELIMINARY MILKINGS.—All tests require a preliminary milking. In taking a preliminary milking the supervisor shall make certain the cow is milked dry. The exact time that this milking is made should be recorded in the barn book. The last milking of the test shall be made neither earlier nor later than the time of the preliminary milking. All preliminary milkings shall be weighed, tested, and reported and shall be considered as one of the regular milking periods with respect to the number of milkings supervised daily.

* With revisions made and adopted by American Dairy Science Association to Dec. 31, 1940. Revisions are indicated by **.

7. RULES FOR CONDUCTING THE TEST.**—Before each milking period, the supervisor shall observe that the milk pail is clean and dry and shall carry the milk pail at all times during the milking period, allowing the pail out of his hands only during the actual milking process.

Where the cows are milked by hand, only one cow may be milked at a time if in a box stall or individual quarters. Two, however, may be milked at the same time if milked by machine or if standing side by side in the stanchion line.

The supervisor must in every case be in a position to observe the milker during the entire milking process.

Milking Machines.—In case a single unit milking machine, tended by one operator, is used, two cows may be milked at a time, but no second man as stripper is allowed. The operator may strip a third cow while two cows are being milked by machine providing the cow being stripped by hand and the two cows being milked by machine are in close proximity. Double units, if used, must be operated as singles. The supervisor must keep the milking machines under as close watch as he would the person doing the milking. The supervisor shall see the tubes of the machine rinsed, and the machines put together previous to milking to make sure that they contain nothing which will affect the accuracy of the sample. The machines are to be run idle for a few minutes before being attached to the cows. The supervisor shall keep the machines under constant watch while being changed from one cow to another. He must be in sight of the machines while weighing and sampling.

Combine Milkers.—Where a combine milker is used samples may be drawn directly from the milk holder of the machine. Samples for testing may be drawn only after the milk in the container has been thoroughly agitated by releasing the vacuum in the container by admitting air through the petcock in the bottom of the container. The attendant must operate the machine so as not to release the vacuum when the milker is removed from the cow. Not more than four units of one section may be supervised at one time and but one attendant is permitted to these units. Scales on each milking unit must be found accurate by the supervisor previous to their use, and he must note that the weighing indicator points to zero before the unit is attached to each cow.

No Conversation During Milking.—The supervisor shall refrain from unnecessary conversation during the time the cow is being milked.

Right of Search.—The supervisor has the right to search the milker at any time. Refusal on the part of the milker will be construed as evidence of intent to make a fraudulent test.

Weighing Milk.—The supervisor shall weigh the empty pail before each milking. Immediately after each milking is completed he shall take charge of the pail and weigh the same on scales provided by the College and enter

the exact weight of the milk at once in his barn book, and see to it that the milker records the same weight on the owner's barn milk sheet. Any inaccuracy in the owner's scale shall be reported to the superintendent in the state concerned and to the breed association.

Sampling Milk.—The supervisor shall take a sample of the milk, being careful that the milk is thoroughly mixed. Such samples shall be kept under lock and key or in the supervisor's sight until the samples have cooled to ordinary room temperature, which is 60 to 70 degrees F. When the last samples at night are kept until morning, unless thoroughly cooled in warm weather, preservative should be added to each sample. These cooled samples shall be warmed to 70 degrees F. before samples are taken for testing.

Applying Babcock Test.—Fat determinations may be made singly. All fat determinations shall be accurately made, giving clear fat columns, or repeated until clear, well defined fat columns are secured. In case samples are tested in duplicate, or where more than one clear test is obtained on a sample of milk the average of all such tests made on that sample must be used in computing the amount of fat. If duplicate determinations vary more than .2 of one per cent the test must be repeated. Readings of the tests shall be made at a temperature of 130–140 degrees F. This temperature is to be obtained by placing the bottles in a hot water bath for 5 minutes. The samples taken at any one milking shall not be thrown away until satisfactory tests of the milking are obtained.

Testing of Acid for Adulterants.—The supervisor shall run a blank test on all sulphuric acid, consisting of 17.6 cubic centimeters of acid and sufficient water to fill the bottle.

8. LOST MILK AND LOST SAMPLES.—No substitution of lost milkings or lost samples is allowed. Any missing data due to loss of milk weights of test samples are to be left blank on the report.

9. PRELIMINARY REPORTS.—The supervisor shall report to the Superintendent of Official Testing any obvious violations of the rules of the College or the breed associations on the part of the owner or attendants, and shall report immediately any cows that meet retest requirements and note upon his report form any sickness of a cow or other conditions likely to affect the reliability of a test.

A preliminary report card shall be mailed by the supervisor from the farm to the breed association immediately after the test. Any cow not in normal condition should be reported on this card.

10. REPORT COWS WHICH ABORT WHILE ON TEST.**—If a cow on official test aborts while in milk, and the gestation is 152 days or longer, her current record shall end and a new lactation shall begin, but if the abortion shall occur at less than 152 days the test is to be continued to complete the 305 day or 365 day test period.

11. USE OF THYROXINE OR THYROID EXTRACTS.**—The feeding or injection of thyroid extract or thyroxine to cows on official test is prohibited and evidence of its use shall be cause for cancellation of such records.

12. REPORTS.—The supervisor shall make out his report so as to give all of the information asked for and then sign and mail them promptly to his appointing officer for checking and endorsement.

13. TEST PERIODS EXCEEDING TWO DAYS.—Where a test is conducted longer than the regular two days, or where more than one test is made in a month, all details of each test from beginning to end shall be reported.

14. PAYMENT OF SUPERVISORS.—Under no circumstances shall any payment, gift, or gratuity to the supervisor be made by, or permitted from the owner of the cow or anyone interested in her, and any violation of this rule will invalidate the test.

15. SUPERVISORS TO SUPERVISE TEST.—The supervisor appointed to conduct a test is the direct representative of the Superintendent of Official Testing and should be respected accordingly. His duties are to supervise tests only.

16. SWORN STATEMENTS.—Each supervisor is required to fill out a sworn statement covering all his work, which eliminates the necessity of his affirming test reports.

17. HEALTH OF SUPERVISORS.**—Supervisors conducting inspections on herds entered on official test shall be required to possess health certificates conforming to the same rules regarding health as required of milkers and other employees in dairies producing milk under regulation of the Standard Milk Ordinance, United States Public Health Service.

18. RESPONSIBILITY FOR ENFORCEMENT OF RULES.—Owners and persons in their employ, are held equally responsible with the supervisor for the enforcement of the foregoing rules. Test reports will not be accepted unless all of the above rules have been observed by all parties concerned.

American Dairy Science Association Announcements

THIRTY-SIXTH ANNUAL MEETING

UNIVERSITY OF VERMONT

Burlington, Vermont, June 23–26

Come to Vermont, the Green Mountain State, which is this year holding its Sesquicentennial celebration. Come by motor, rail or air, but be sure to come. Bring the family. Special entertainment is planned for the ladies and children. Everyone will enjoy the Green Mountains. Burlington is beautifully located on historic Lake Champlain and the University commands a wide expanse of unexcelled views of mountain, lake and valley. To the west across the lake are the Adirondacks, a perfect background for gorgeous sunsets. Many will enjoy boatrides, swimming, golf and tennis.

A reservation form and information relative to housing facilities have been sent to each member. Anyone who has not received this material or who desires same should make request to J. M. Frayer, chairman of registration committee, 489 Main Street, Burlington, Vermont.

It is our desire to make this visit to Burlington and Vermont both pleasant and profitable. The prompt filling out and return of the reservation form will greatly assist us in doing the things we would like to do for your comfort and entertainment. If you have not already attended to this matter, do it today, please.

Choice of several kinds of accommodations is available to those who make advance reservations. There are hotels, college dormitories, private homes, tourist cabins, etc.

As previously announced, all those who will attend this 36th Annual Meeting are cordially invited to visit colleges, experiment stations and other points of interest in states and provinces surrounding Vermont.

Desiring to be of service especially to those who will come by automobile, Ontario, Quebec, New Hampshire, Massachusetts, Connecticut, New Jersey and New York have all joined Vermont in preparing brief lists with locations of outstanding breeding establishments, dairy plants, and points of historic or scenic interest. These lists are now being arranged and prepared for distribution to all who signify by advance registration that they are coming.

VOLUME XXIV MAY, 1941 NUMBER 5

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

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Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
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THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

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New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

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The Royal Technical College, Copehagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION IN THE JOURNAL OF DAIRY SCIENCE

304. Factors Affecting Activity and Heat Resistance of Swiss Cheese Starter Cultures. III. Effect of Variations in Time and Temperature of Incubation and of Storage on Activity of Cultures. H. J. PEPPLER AND W. C. FRAZIER, Department of Agricultural Bacteriology, University of Wisconsin.

The dairy manufacturer frequently wonders how much he can vary methods of preparation and storage of lactic acid bacteria without loss in their vitality. The results of this study indicate that incubation time and temperature can be varied considerably without reduction in the activity of certain starter organisms for Swiss cheese.

After numerous transfers in reconstituted skimmilk at controlled times and temperatures, the activity of *Lactobacillus helveticus* and *Streptococcus thermophilus* was determined by means of direct microscopic counts and measurement of acid production in 12-hour bulk cultures.

Cultures of *L. helveticus* were equivalent in activity after numerous transfers every 12 to 24 hours at 37°, 40° and 42° C. Similar treatments of *Str. thermophilus* produced cultures of equivalent and maximum activity when successive transfers were made at 37°, 40°, 42° and 45° C. After incubation at 37° from 12 to 16 hours or at 42° for 12 to 24 hours, cultures of *L. helveticus* and *Str. thermophilus* could be stored at 0° or 20° C. until they were 96 hours old without reduction in growth and acid production.

The effect of these same variations on the heat resistance of cultures will be discussed in a succeeding paper.

305. Factors Affecting Activity and Heat Resistance of Swiss Cheese Starter Cultures. IV. Effect of Variations in Time and Temperature of Incubation and of Storage and Heat Resistance of Cultures. H. J. PEPPLER AND W. C. FRAZIER, Department of Agricultural Bacteriology, University of Wisconsin.

Previous studies had indicated that incubation periods at different constant temperatures near the optimum for growth, or such periods followed by storage at low temperatures, could be varied considerably without reduction of vitality of *Streptococcus thermophilus* and *Lactobacillus helveticus*. The effect of similar variations on the heat resistance of both organisms was studied by methods described in the first paper of this series.

A variety of modifications can be applied to procedures for the preparation of *L. helveticus* and *Str. thermophilus*, depending upon incubation tem-

perature employed, storage temperature available, and quality of culture medium selected, without decreasing the activity and heat resistance of cultures. Cultures of *Str. thermophilus* with equivalent and maximum heat resistance were obtained when transfers were made every 12 and 24 hours at 37° C., or initial incubation periods of 12 hours were followed by storage at 12° for as long as 36 hours. Enrichment of the skimmilk culture medium with Neopeptone markedly enhanced vitality of the cultures, and resulted in the most heat resistant cultures when incubation was at 37° and 40° C. Such cultures held 10 to 12 hours at 37° C. could be stored for 84 hours at 12° C. without reduction in activity and heat resistance. Results with *L. helveticus* were, in general, similar.

Association with *Candida krusei*, a film yeast, increased the heat resistance of these lactic acid bacteria.

Practical applications of the results are suggested.

306. In vivo Studies of Hydrogen Ion Concentrations in the Rumen of the Dairy Cow. VEARL R. SMITH, Department of Dairy Husbandry, Oregon State College.

A study was made on two rumen fistula cows of the pH of rumen ingesta. The pH readings were made *in vivo* with a Beckman pH meter and a glass electrode assembly constructed for such purposes. Readings of pH were taken three times a day over a period of five days and at two-hour intervals over a period of 24 hours for alfalfa hay and alfalfa hay plus beet pulp rations. A mean hydrogen ion concentration of 6.27 was obtained for alfalfa hay alone and a pH of 6.00 for alfalfa hay plus beet pulp for three-times-a-day readings over a period of five days. Comparisons were made of *in vivo* and *in vitro* pH determinations. Lower pH values were obtained by the *in vivo* method.

307. An Analysis of Contestant Judgments in the Scoring of Dairy Products with a Study of Some Factors Which May Affect Them. G. M. TROUT, CH., WILLIAM WHITE, P. A. DOWNS, M. J. MACK AND E. L. FOUTS, Committee on Judging Dairy Products, A. D. S. A.

A critical study was made of 2520 sample judgments in the scoring of butter, cheese, milk and ice cream in the 1940 Students National Contest in the Judging of Dairy Products, involving 2520 item judgments for butter and 1890 each for cheese, milk and ice cream, a total of 8190. The 63 contestants, assigned to four groups, judged the four products during four periods of 55 minutes each.

The two groups scoring butter and ice cream in the first period had slightly lower grades than the groups which scored those products in the second, third, or fourth periods. On the other hand the three groups which

scored cheese after having scored milk had a much lower grade than the group which scored cheese first. The best judging of milk was done in the third period.

Excitement and fatigue appeared to be factors of little consequence as indicated by the total scores of the various periods. Slightly lower grades, showing superior judging, were obtained in the third and first periods respectively; the highest grades, showing poorest judging, being made during the second period.

Higher average grades were made on scoring flavor of milk and ice cream than on scoring flavor of butter and cheese, whereas, little difference was noted between the average grades on criticisms for the four products.

Contestants appeared to be more conservative in scoring strong off-flavors of milk and ice cream than the official judges.

The change in body and texture of the products during the progress of the contest as affecting its scorability would seem to be of minor importance, dependent upon the product; changes in cheese being of little consequence; changes in ice cream negligible, except possibly for melting quality; whereas, changes in body of butter were such as to increase slightly the average contestants grades per period of judging.

308. The Limit of Error of the Simplified Vacuum Solids Test as Applied to Ice Cream Mix, Evaporated and Sweetened Condensed Milk. L. G. HARMON AND K. M. RENNER, Texas Technological College, Lubbock, Texas.

A new test has been devised for the determination of total solids in ice cream mix, evaporated and sweetened condensed milk. This test has been called the Simplified Vacuum Solids Method.

The apparatus for performing total solids analysis by this method can be assembled at low cost from ordinary laboratory glassware.

In order to determine the accuracy of this testing device, groups of fifty total solids analysis were made on each of the above mentioned products, and the results were compared to analyses made on the same products by the Official Method.

A statistical analysis of the resulting data was made. The results compare favorably with the results obtained by other workers, who measured the accuracy of other commonly used methods.

309. The Relationship of Fat to Quality, and Methods of Standardizing the Fat Content, in Swiss Cheese. G. P. SANDERS, R. R. FARRAR, F. FEUTZ AND R. E. HARDELL, Division of Dairy Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture, Washington, D. C.

Factors responsible for variations in quality and composition of Swiss

cheese have been studied in 39 factories, in cooperation with the Departments of Dairy Industry of the University of Wisconsin and Ohio, and in the laboratories of the Bureau of Dairy Industry. Results already published are described in the JOURNAL OF DAIRY SCIENCE, *19:* 395–404. 1936; *22:* 43–48. 1939; *23:* 905–18. 1940.

Tabulations of analytical and grading data on 844 wheels of factory Swiss cheese show that those containing between 45 and 46 per cent fat in dry matter were of the highest average quality, and that those containing more than 48 per cent were of higher average quality than those containing less than 43 per cent. In controlled comparisons on 30 pairs of laboratory cheese made from high-solids milk, and therefore rather firm in texture, those containing an average of 48 per cent fat in dry matter were superior to those containing less than 45 per cent. Low fat content was generally associated with high moisture content, which led to defects in flavor and eye formation.

For securing results on composition promptly without plugging the cheese, equipment was devised for mechanically pressing small samples of kettle curd, analyses of which provided a means of predicting percentage of fat in dry matter in the cheese. In about 500 pairs of samples, values of fat in dry matter of pressed curd were within 1 per cent of those found in the corresponding cured cheese in 70 per cent of the cases, and within 2 per cent in 96 per cent of the cases.

Fat tests in kettle milk can be used to predict percentages of fat in dry matter in the cheese, but results by this method alone are less precise than by the pressed-curd-analysis method. Higher fat tests in kettle milk are required during the fall and winter months than in summer, to produce uniform standardization of cheese. Standardization procedures yield more precise results in any one factory than is indicated by data for numerous factories located in different areas.

A slight decrease in percentage of fat in dry matter which occurs during curing of Swiss cheese can be accounted for by the increase in dry matter resulting from the absorption of salt.

For efficient standardization it is necessary that the milk in every kettle be tested for percentage of fat, and it is recommended that samples of pressed curd and of the corresponding cured cheese be obtained frequently and analyzed.

310. **The Significance of Lipolysis in the Curd Tension and Rennet Coagulation of Milk. I. The Role of Fat Globule Adsorption "Membrane." II. The Effect of the Addition of Certain Fat Acids to Milk.** N. P. TARASSUK AND G. A. RICHARDSON, Division of Dairy Industry, University of California, Davis, California.

Milks and their creams whose normal fat globule adsorption "mem-

brane'' material is replaced by other surface-active material through re-emulsification of the milk fat will exhibit a pronounced lipolysis of fat if their source of milk plasma is raw skim milk. An extensive lipolysis of milk fat takes place, for example, when gelatin, whey powder, calcium caseinate, skim milk powder, or skim milk itself are used as the emulsifying agents. When a pasteurized skim milk is substituted for a raw skim milk as the source of milk plasma, the lipolysis is not evident.

The paper discusses the role of natural fat-globule-adsorption ''membrane'' in relation to lipolytic activity of normal milk, and advances the theory that the natural adsorption layer on fat globules affords protection from the lipolysis of milk fat.

The lipolysis of milk fat in a natural or synthetic cream may resolve into reduction of curd tension or into a complete inhibition of rennet coagulation of buttermilk obtained by churning a natural or synthetic cream. This effect is due to the interference of high melting-point fat acids with the normal action of rennet on milk. Evidence is submitted that the direct addition of lauric, myristic, or palmitic acid to milk completely inhibits the rennet coagulation, thus reducing the curd tension of milk to zero provided the conditions as to the amount of acid added and the aging of milk in the cold thereafter are satisfied. The addition of $CaCl_2$ to milk nullifies the inhibitive effect of fat acids in rennet coagulation of milk. The normal rennet coagulation and curd tension of milk are also restored by the heat treatment of milk sufficient to change the physical state of the fat acids involved from solid to (or near to) the liquid state. The restoration of normal milk properties by the heat treatment provides a basis for speculation concerning the mechanism whereby certain fat acids inhibit the coagulation of milk by rennet.

311. Effect of Humidity on Moisture Content and Forms of Lactose in Dried Whey. PAUL F. SHARP AND HUGO DOOB, JR. Department of Dairy Industry, Cornell University, Ithaca, New York.

A series of wheys containing either alpha hydrate or beta lactose anhydride as crystalline solid phase were exposed to atmospheres of several constant relative humidities. The wheys did not absorb excessive amounts of moisture at relative humidities below 40–50 per cent.

Between 30 and 50 per cent humidity wheys, if held long enough, absorbed and subsequently rejected moisture. This was accompanied by slow continued crystallization of that form of lactose initially present as a crystalline phase. In this way either beta lactose or alpha hydrate crystallized at room temperature.

Above 65 per cent relative humidity and at 25° C. crystalline beta lactose present in certain types of whey was transformed to the less soluble alpha hydrate.

Variation in temperature at 55 per cent relative humidity served only to hasten crystallization and the attainment of moisture equilibrium.

The equilibrium moisture content of dried wheys at constant relative humidity below 65 per cent was found to be greater for those containing alpha lactose hydrate than for others containing beta anhydride as the solid modification. The difference was largely the water of crystallization of the sugar.

The lower the percentage of lactose present in the dry matter the greater the moisture content of the dried whey at constant relative humidity. This was attributed to the osmotic activity of lactic acid produced by fermentation.

312. The Effect of Curd Tension of Milk on the Utilization of Added Vitamin D. W. E. KRAUSS, T. S. SUTTON, L. H. BURGWALD, R. G. WASHBURN AND R. M. BETHKE. Departments of Dairy and Animal Industry, Ohio Agricultural Experiment Station, Wooster, Ohio.

Normal milk, homogenized milk and mineral modified soft curd milk, all from the same source, and natural soft curd milk were pasteurized and fortified to the extent of 400 U.S.P. units of vitamin D per quart. These milks varied in curd tension from 0.0 grams for the mineral modified milk to 54.9 grams for the normal milk. Bioassays of these fortified milks by the line test procedure resulted in the same degree of healing when equal amounts of each were fed. In a prophylactic trial equal intakes of the fortified milks resulted in almost identical bone ash values.

It is concluded that the effectiveness of added vitamin D is not influenced by the curd tension of milk.

313. Quantitative Determination of Alpha and Beta Lactose in Dried Milk and Dried Whey. PAUL F. SHARP AND HUGO DOOB, JR. Cornell University, Ithaca, New York.

A clear colorless extract of the product, obtained by clarifying with alcoholic mercuric chloride and decolorizing with norrit, was polarized at once, at ten successive minute intervals, and finally after equilibration. The relative amounts of alpha and beta lactose were calculated from the initial rotation extrapolated to the time of dissolving the sample, and the final rotation; total anhydrous lactose from the final rotation, sample weight and dilution. The anhydrous lactose in dried whey was found to exist from 90 per cent in the beta, to 95 per cent in the alpha form.

Specific rotations at 25° C. and 589 μ were anhydrous alpha (weighed as hydrate) + 89.5°, beta + 35.0° and equilibrium mixture + 55.1°. Norrit was found to adsorb lactose from solution, the alpha form being adsorbed preferentially. Lactose was eluted by 10 to 20 per cent alcohol.

314. Evidence for the Presence of Smooth Muscle Elements Surrounding the Alveoli of the Mammary Gland. ERIC W. SWANSON AND C. W. TURNER. Department of Dairy Husbandry, University of Missouri.

The udder of a cow in advanced lactation was examined histologically to investigate the cells surrounding the alveoli. Suitable staining revealed cells beneath the secretory epithelium of the alveoli and in the interlobular connective tissue which had the appearance and staining properties of smooth muscle cells. Photomicrographs are presented clearly showing the different cell types. The smooth muscle cells were observed spaced at intervals around the alveoli and along the walls of the ducts. It is believed that they function in the expulsion of milk by contracting the alveoli and ducts and forcing the milk toward the cisterns.

BOOK REVIEWS

315. The Chemical Action of Ultra Violet Rays. CARLTON ELLIS, ALFRED WELLS AND FRANCIS F. HEYROTH. 961 pages, published by the Reinhold Publishing Corp., 330 West Forty-second Street, New York, N. Y. $12.00.

This review and text consists of 42 chapters and is divided into 5 parts (1) an introduction on the nature of ultra violet radiation, (2) 10 chapters on the various sources of ultra violet radiation, (3) 17 chapters on the photo chemical processes including both organic and inorganic reactions, (4) 5 chapters on applications of photo chemistry to industrial products and (5) 11 chapters on applications of ultra violet rays in biology.

The subject material covers a number of phases of real interest to those in research in the dairy industry. Among these may be cited: the transmission of glasses and filters used in analytical research; the variation of solar radiation; a review on the effect of radiation on the fats and oils in various food products, although that pertaining to milk fat and butterfat seems inadequately reviewed; reference is made to the sterilization of milk and other opaque substances, but the bacteriology of specific food products is not reviewed, although the lethal action of the radiation on microbial life is applicable to the irradiation of foods.

The text contains a 69 page excellently compiled review on the subjects of: rickets and irradiation, photo chemistry of the formation of vitamin D, the commercial production of vitamin D, and of vitamin D milk. Other subjects of interest to those in dairy research include discussions on the effects of ultra violet on visual purple, the flavins, other vitamins, enzymes, proteins, and the sterilization of water for food supplies. The mechanics of the various sources of ultra violet radiation adaptable for commercial and research work is well covered.

This book contains no reviews on the effects of the visible fractions of radiation on milk and milk products, nor was it intended to do so, although a review of this phase is to be desired by those in the dairy industry in view of known effects of visible radiation on milk. The text contains a very useful detailed and complete author and subject index and foot note reference list throughout. Because of the increasingly evident effects of radiation upon the properties of milk including flavor, color, and nutritional value, and the increasing use of radiation in the analysis of milk products, as well as the processing of food, and sterilization of air and water, the reviews in this text, in view of their scope, must certainly be of real value to the research laboratory. It provides information in compiled form that has long been needed. K.G.W.

316. Diseases Transmitted from Animals to Man. THOMAS G. HULL. 2nd edition, 1941. 403 pages. Published by Charles C. Thomas, Springfield, Ill. $5.50.

This book represents a concise, readable summary of otherwise widely scattered information. The material is classified in five sections, *Diseases of Domestic Animals and Birds, Rodent Affections, Human Diseases Spread by Animals, Animals as Passive Carriers of Disease Organisms, and A Review of the Role Played by Each Animal in the Spread of Disease.* These comprise 32 chapters of which the first 31 are devoted to as many diseases. Since publication of the first edition in 1930 the original chapters have been reviewed and revised by 18 specialists who are recognized as authorities in their respective fields. New chapters have been added on louping ill, sore mouth of sheep, equine encephalomyelitis, Rift Valley fever, typhus fever, and relapsing fever.

Subheads within each chapter provide ready reference use of the material and serve to indicate the scope of treatment, as in Chapter I, Tuberculosis: History, Types of Organisms, Susceptibility to Infection, Bovine Tuberculosis, Human Infections with Bovine Tubercle Bacilli, Transmission of Tubercle Bacilli through Milk, Transmission of Tubercle Bacilli through Meat, Avian Tuberculosis, Tuberculosis in Swine, Prevention and Control, Eradication of Tuberculosis in Animals, Pasteurization, Meat Inspection, and Immunization against Tuberculosis. Controversial discussion is avoided although facts in disagreement may be presented. Especially useful are statistical data pertaining to incidence and distribution of the diseases. Each chapter is terminated by a brief, enumerated summary, titled "Items of Note," and an extensive bibliography.

While this book serves best the veterinarian, the public health official, and the laboratory worker and is especially invaluable in public health education, there is much of interest to dairy husbandry and the dairy industry. Chapters that will appeal to the dairy worker are those on Tuberculosis,

Anthrax, Foot-and-Mouth Disease, Brucellosis, Milk Sickness, Smallpox and Cowpox, Rabies, Food Infections, Fungous Diseases, The Relations of Human Infections to Animals, Septic Sore Throat, Diphtheria, Scarlet Fever, and The Role of Cattle . . . Goats . . . (and other animals). Proper consideration is allotted to milk borne diseases and to the importance of pasteurization. The index lists 32 references to diseases in cattle and 18 in goats. Under "Milk" are found "transmission of diphtheria bacilli," "poliomyelitis virus," "rabies virus," "streptococcus and scarlet fever," "streptococcus of septic sore throat," "typhoid bacilli," and "tubercle bacilli."

Forty-five illustrations implement the text. The index is well organized. Typography and construction are excellent. For those interested in disease transmission by dairy animals and milk a more useful work will be hard to find. D.P.G.

BREEDING

317. Ein neuer Letalfaktor beim Rinde. D. Ilancic. Züchtungskunde, 15: 5; 129–133. 1940.

A bull of the gray brown (Montafoner) mountain race in Croatia sired 33 normal calves and 37 calves whose nostril openings were grown together and whose skulls were more or less malformed. All these defective calves were dead at birth or died soon afterward. Photographs of three heads are shown. A possible Mendelian explanation is proposed. J.L.L.

318. Improved Herd Efficiency Through Artificial Breeding of Dairy Cattle. J. W. Bartlett. Certif. Milk, *16:* 177; 7. Jan., 1941.

Four important essentials appear to be necessary to the successful operation of a cooperative artificial breeding association. They are:

1. An intense interest in herd improvement by the members and enough cows enrolled to insure sufficient finances.
2. Exceptional sires available for use.
3. A competent technician, preferably a veterinarian.
4. Reasonable patience.

It is pointed out that cooperative artificial breeding offers the dairyman a way to decrease his costs. W.S.M.

BUTTER

319. The Quality of Butter Made from Vacuum-pasteurized and Vat-pasteurized Lots of the Same Creams. N. E. Fabricius and E. W. Bird. Iowa State College Res. Bull., 284. Nov., 1940.

Gathered cream was neutralized 0.25 or to 0.15 per cent acidity and then vat pasteurized at 155° F. for 30 minutes or pasteurized in the "Vacreator"

at 190 to 204° F. under vacuum. The butter was scored fresh, after 6 weeks at 38° F., and after 6 months at 0° F.

The butter made from vacuum-pasteurized cream scored higher than the butter from vat-pasteurized cream when fresh and after storage. This difference was true throughout the year but was especially significant during the summer months when the cream was weedy in flavor. In the winter months the body of the butter from vacuum-pasteurized cream was noticeably improved.

There was no significant differences in the bacterial, mold or yeast counts of the butter. The vacuum-pasteurized cream was sometimes phosphatase positive. A.C.D.

320. A Visual Mold Test for Cream and Patron Reaction to It. C. H. PARSONS. Nat. Butter and Cheese J., *32:* 3; 12. 1941.

This test is a modification of the Wildman technique. The somewhat different reagent causes more clumping of mold, hastens filtration and leaves the clumped "milk-mold" free from curd. This mold is without public health significance. The amount of mold collected can be easily shown to the producer. Nine cc. of well-mixed sample are pipetted into a 2-oz. jar. A 17.6 cc. acid dipper of the reagent solution is added and the mixture is heated for three minutes with gentle agitation and half a minute more with no agitation before it is completely poured through a 1¼-inch organdy disc so mounted in a filtering device that mold is evenly distributed over the filtering surface. Excess liquid is absorbed from the bottom of the disc with a dry cloth and the disc is then ready to compare with American Butter Institute Standards. A six weeks' investigation in Summer, continued for 2 weeks in Fall on 400 farms, at cream stations and on cream routes disclosed that producers reacted favorably to the test by producing better cream after seeing it work. It was found that the test is an index of butter quality; that cream is better when it is produced by mechanical separators and then cooled and stored in a dry place at temperatures below 60° F.; that cans which permit minimum exposure of cream surface to air are better than partly filled cans; that clean production methods maintain lower mold counts; and that chlorine sterilization seems to be more effective than poorly managed hot water treatments. Detailed instructions and specifications for the test are given. W.V.P.

321. Manufacture of High Scoring Butter. N. E. FABRICIUS. Nat. Butter and Cheese J., *32:* 3; 16. 1941.

The value of the acidity test can be easily over-estimated in judging cream quality. Cream samples with specific defects were selected, scored, neutralized, pasteurized and churned. The quality of the resulting butter showed that there is a definite tendency by experienced people to score cream

with specific defects too high, possibly because usual methods of neutralization and pasteurization cause less improvement than is generally considered. Recent experiments at Iowa indicate that high acid cream should not be neutralized below 0.25 per cent acid before pasteurization. It is wise to neutralize from 0.25 to 0.10–0.15 per cent following pasteurization when the cream has been cooled to 120° F. or below. Butter should have a pH of 6.7–7.3 in the serum to have the best keeping quality. Five important points should be remembered in using butter cultures: the culture vat should have adequate temperature control, quick cooling and vigorous agitation; citric acid should be used when citrates are lacking; heavy inoculations should be used to guard against culture failure; the sense of taste and the creatine test are better than the acid test to indicate end of ripening; and adding highly ripened culture to cream gives a more uniform flavor. Leaky body, which can be avoided by correct churning temperatures, washing treatments and working procedures, is associated with the development of stale, putrid and cheesy flavors in stored butter. W.V.P.

322. **Crumbliness and Stickiness in Butter.** G. H. Wilster, I. R. Jones and J. R. Haag. Nat. Butter and Cheese J., *32:* 1; 14; *32:* 2; 16; *32:* 3; 20. 1941.

When butter is cut with a knife and spread on bread it should not crumble and stick to the knife. In Oregon such defective butter is most commonly made from September to May, chiefly in areas where cows get the most dry feed. The problem is important because Oregon butter competes with that from many states. This report discusses effects of feed and season on fat composition and the variations in the chemical and physical properties of fat produced in 3 areas of Oregon.

"When alfalfa hay was fed as a sole ration to cows, the fat of the milk was found to have a relatively high melting point and a low content of the unsaturated and volatile fatty acids.

"When grain was fed in addition to alfalfa hay, there was a decrease in the unsaturated fatty acids as indicted by the iodine number and an increase in the volatile, soluble fatty acids as indicated by the Reichert-Meissl value.

"Pasturing cows in addition to feeding alfalfa hay or alfalfa hay and grain caused a marked decrease in the melting point, a considerable increase in the iodine number, and distinct increases in the Reichert-Meissl value and the refractive index.

"The addition of artichokes to an alfalfa hay ration caused a slight increase in the melting point, a marked decrease in the iodine number, a distinct increase in the Reichert-Meissl value and a small decrease in the refractive index.

"The addition of artichokes to an alfalfa hay and grain ration caused

only a slight change in the melting point, a decrease in the iodine number, an increase in the Reichert-Meissl value, and little change in the refractive index.

"When eastern-Oregon-grown alfalfa hay was fed as a sole ration to a herd of cows, composed of three breeds, at Corvallis, the fat in the milk had a lower melting point and a higher percentage of the unsaturated and volatile components than did the fat produced by a herd of Jersey cows also fed eastern-Oregon-grown alfalfa hay at the Umatilla Field Station.

"Feeding Willamette Valley alfalfa hay, grain and corn silage to a herd of Holsteins, Ayrshires and Jerseys at Corvallis caused the fat in the milk to have a slightly lower melting point, a lower iodine number and a considerably higher Reichert-Meissl value than did feeding eastern Oregon alfalfa hay as the sole ration to a herd of cows composed of the same breeds at the college dairy farm at Corvallis.

"Butter manufactured at an eastern Oregon creamery located in an alfalfa-producing section contained fat that had a relatively low melting point, a high iodine number, a high Reichert-Meissl value and a high refractive index during the Spring and Summer months, and a high melting point, a low iodine number, a low Reichert-Meissl value and a low refractive index during the Fall and Winter months. The butter made during the Winter season showed crumbliness and stickiness in varying degrees.

"Butter made in 15 creameries located in the central, eastern and southern Oregon, Willamette Valley and coast areas of Oregon showed a variation in body and texture characteristics and in the physical and chemical properties of the fat. The butter made in the coast and Willamette Valley areas had a medium-firm body and a satisfactory texture, whereas the butter made in the central, eastern and southern Oregon alfalfa-producing areas had a firm body and generally showed stickiness or crumbliness or sometimes both defects.

"The fat of the butter made in the coast and the Willamette Valley areas had a higher Reichert-Meissl value and a higher Polenske value than did the fat of the butter made in the central, eastern and southern Oregon area. There was no appreciable difference in the iodine value of the fat from the different sections.

"Distinct crumbliness and stickiness were observed in the butter made in the alfalfa-producing sections of central, eastern and southern Oregon when

1. The melting point of the fat was above 33.5° C. (92.3° F.).
2. The iodine number was below 33.
3. The Reichert-Meissl value was below 28.
4. The refractive index was below 1.4536.
5. The Polenske value was below 2." W.V.P.

323. The Cost of Making Butter. G. M. PELTON. Can. Dairy and Ice Cream J., *19:* 9; 14. 1940.

The author gives a detailed account of all the items which make up the cost of a pound of butter. Of particular importance at this time is the cost of increased taxes of all kinds. Many new kinds of taxes have developed in the last few years such as old age pensions, unemployment compensation, etc. O.F.G.

324. "Vacreated" Cream for Buttermaking. R. W. BROWN. Can. Dairy and Ice Cream J., *19:* 9; 26. 1940.

This is a report supplementary to another one reported earlier in this journal. The vacreated butter scored higher in flavor than the pasteurized butter in 173 cases out of a total of 196 trials or 88.2 per cent of the cases. O.F.G.

325. Print Butter Discoloration. A. H. WHITE. Can. Dairy and Ice Cream J., *19:* 10; 19. 1940.

Defects in color of print butter may be of two kinds: 1. Those caused by poor workmanship which show up as uneven, mottled or streaky color; 2. Those of a microbiological or chemical origin which are due to the growth of various types of mold or color-producing bacteria, or chemical changes in the butter components. Color defects of the first kind are easily controlled by careful attention to manufacturing procedures. Pigment-producing organisms have caused some annoyance and loss of Canadian butter which had been mildly salted. An organism capable of producing a bluish-black pigment at temperatures between 40° and 60° F. in low salt concentration was isolated. The defect could be reproduced by inoculating cream before churning or the butter with infected wash water. Studies indicated that the organism belongs to a group usually associated with soil or water and the name *Pseudomonas nigrifaciens* was suggested. Practical control measures should include thorough sterilization of equipment, general plant sanitation and salting the butter to contain not less than 1.25 per cent salt. O.F.G.

326. A Discussion of Buttermilk Testing Methods. E. W. BIRD. Can. Dairy and Ice Cream J., *19:* 10; 52. 1940.

Buttermilk testing has received considerable attention because (1) buttermilk losses play a part in balancing fat receipts against fat outgo and (2) a statistician can amass a sizable figure in dollars if aggregate fat losses are considered. About 75 per cent of the fatty materials in buttermilk are true fats and 25 per cent consist of sterols and phospholipins. The sterols and phospholipins are purchased as fat because they are included in the Babcock test or the Mojonnier test. The kind of test used does not matter so

long as (1) the reading is relatively large and (2) the correct interpretation is placed upon the results. The Minnesota A test and the American Association (butyl alcohol) test are recommended because they give relatively large readings. A simple method for evaluating loss of fat in churning is given. O.F.G.

327. The Distribution of Surplus Butter. CHARLOTTE I. JOHNSON. Can. Dairy and Ice Cream J., *19:* 11; 68. 1940.

During the early months of 1939 stocks of butter in storage in Canada were greatly in excess of domestic requirements. Through the action of the Dominion Parliament and under the supervision of the Red Cross, vouchers for butter were issued to relief recipients in units of 1 to 7 pounds and to non-relief recipients in units of 1 to 3 pounds. Households receiving free butter substantially increased their consumption of butter over the amount used previously although the purchases during the time of free distribution, particularly among those on low wages, did not equal those made before the distribution of surplus butter. O.F.G.

328. Fats and Oils Battle for Food Industries Market. GORDON W. MCBRIDE. Food Indust., *12:* 2; 60–63. 1940; *12:* 3; 70–73. 1940.

The principal oils commercially, are: edible—butterfat, lard, cottonseed oil, and oil from corn, soybeans, peanut, etc.; inedible—tallow, grease, marine oil, linseed oil, tung oil, palm oil, etc.

Competition exists as economic, political and technologic.

Butterfat has to compete with hydrogenated oils in margarine and to some extent as a fat used in baking. Economically, butterfat is at a disadvantage but thus far politically, butterfat is protected. The margarines are still looked upon as substitutes and therefor inferior and the dairy interests have succeeded in having margarine heavily taxed. Technologically, butterfat and the hydrogenated oils are nearly equal.

Due to research work each oil is constantly either gaining or losing, never standing still.

This article also gives a great many valuable tables. J.C.M.

329. Steam Deodorization of Edible Fats and Oils. Theory and Practice. A. E. BAILEY. J. Indust. Engin. Chem., *33:* 3; 404–408. 1941.

It is common practice to render edible oils odorless and tasteless by a process of steam distillation under reduced pressure. The process is widely used in the United States for converting cottonseed oil to edible purposes. In this paper the theory of the process is considered in some detail and experimental data are given on the operation of commercial equipment. The odoriferous substances are present in such oils as cottonseed, soybean,

palm, tallow and lard in concentrations of about 0.20 per cent. The nature of the substances is unknown but they appear to be aldehydic and ketonic in nature and their removal parallels that of the free fatty acids.

The author discusses the theory of steam distillation and the vaporization efficiency to be expected in practice. Losses from the deodorizer increase with increases in operating temperature but the time required for deodorization shows a marked decrease as the temperature is raised. Entrainment losses are of considerable importance. High vacuum is employed to decrease steam requirements. B.H.W

CHEESE

330. The Ripening of Cheese. A. Wojtkiewicz and G. Inikkoff. Food Manufacture, *15:* 6; 160. 1940.

This article contains the results of experiments on the speeding up of the ripening of cheese.

Various chemicals such as lactose, lactic acid, phosphates and carbonates, alone and in combination were introduced into the milk and into the cheese. Some of them affected the physical condition of the cheese, some checked the ripening and those which increased the degree of ripeness, the citronates, affected the quality. There appeared to be no application developed from these trials.

The introduction of ferments such as pepsin, tryspin and rennet, addition of autolytic agents such as yeast and *Streptococcus lactis*, and the addition of selected stocks of lactic acid bacteria were tried. The selected stocks were made by cultivation on a medium of chalk to eliminate the harmful influence of surplus acid and were cultivated at low temperatures for a prolonged period.

Cheese made from pasteurized milk to which was added 0.4 per cent of bacteriological yeast using the selected stocks of lactic acid bacteria reached at one month the ripeness normally obtained in 2 to 2½ months.

The use of certain combinations of selected stock of *Streptococcus lactis* and of *B. casei* and yeasts also accelerated ripening and improved the taste. J.C.M.

331. The Need for Uniform Dairy Legislation. George L. Mooney. Nat. Butter and Cheese J., *32:* 3; 17. 1941.

The problems of the dairy industry today are national in scope. Lack of uniformity in dairy laws encourages competitive evils which are especially apparent in cheese laws pertaining to fat, moisture, and labeling. The Federal act specificially exempts butter, cheese and ice cream from declaring the presence of added color on labels but all states have as yet not followed this lead. Since this use of color is not to deceive the consumer, conceal

defects or to imitate another product, it should be permitted and state laws should be drawn to conform to the Federal regulations. W.V.P.

332. Suggestions on Advertising Cheese. C. R. BARKER. Nat. Butter and Cheese J., *32:* 2; 54. 1941.

The most powerful selling force is the merit of the product. Advertising is a process of education based on a good product, a good sales force and good distribution. Good advertising will arouse interest and create a desire to buy. It will not make impossible claims. Its cost should be a direct charge against the product. W.V.P.

333. Flavor Development in Pasteurized Milk Cheese. W. C. HARRIS AND B. W. HAMMER. Can. Dairy and Ice Cream J., *19:* 9, 17. 1940.

Adequate pasteurization of milk for cheese insures destruction of pathogens and tends to control objectionable fermentations. Cheddar cheese made from pasteurized milk, however, does not develop the characteristic flavor normally found in raw milk cheese of fine quality. The work reported here deals with the effect of various bacteria of the genus Micrococcus and the genus Propionibacterium on flavor of cheese made from pasteurized milk. Of the 34 cultures of micrococci studied 7 had an undesirable effect, 14 had no definite effect and 13 had a favorable effect on cheese flavor. Of the latter group, flavors more or less characteristic of well ripened cheddar cheese developed. Certain strains of propionic acid bacteria, when used in relatively small numbers improved the flavor of pasteurized-milk cheese while others had no definite effect; when used in large numbers all the strains improved the flavor. O.F.G.

334. Some War-time Problems in Cheese-making. J. G. DAVIS, England. Can. Dairy and Ice Cream J., *19:* 9, 52. 1940.

The outlook of the cheese-maker is conditioned by the food policy of the government during war. For instance, "How much cheese am I to make?" and "What type of cheese is required?" A slight reduction in milk production does not affect the cheese-maker since his occupation is seasonal. Waste of milk must be avoided. Cheese, therefore, can be regarded as a palatable concentrate of first-class food and the cheese-maker as a conserver of the more valuable part of the milk. Utilization of whey as human food is coming into prominence. Whey may be used as a source of lactose which in turn may be converted to fat or protein by microorganisms or fermented to lactic acid. Excellent grass silage can be made with whey which is nearly as valuable as fresh grass for feeding purposes. The high temperature-short time process of pasteurization (162° F. for 15 seconds) is recommended for factory cheesemakers. Testing all milk for mastitis is recommended in order to avoid cheesemaking trouble. O.F.G.

335. Mold Inhibitors for Cheese. O. R. Irvine and W. A. Sproule. Can. Dairy and Ice Cream J., *19:* 11, 19. 1940.

Four different molds of the Penicillium type which had shown considerable resistance to the effects of propionate salts were used to inoculate the surfaces of cheese slices which had previously been dipped in solutions of propionates or propionic acid. Propionic acid was considerably superior to the salts while the calcium salt was inferior to the sodium salt. Calcium propionate solutions on the cheese resulted in a much less intense flavor than either propionic acid or the sodium salt. A mixture of 10 per cent calcium propionate and 10 per cent propionic acid in the ratio of 5 to 1 provides an immersing agent of a pH of about 5 and has superior inhibiting power. It can be used on highly flavored cheese without imparting a foreign flavor but may impart some flavor to a mild cheese. O.F.G.

CONCENTRATED AND DRY MILK; BY-PRODUCTS

336. Success in By-product Production Demands Quality Control. Joseph Chrisman. Food Indust., *12:* 8; 46–47, 96. 1940.

The production of a good quality casein which will be uniform over a period of time in order to meet the competition now existing is described. This illustrates the fact that in the manufacture of any by-product rigid control is necessary to give the quality which will sell the best.

In the production of casein the main features are final "free" acidity, total acidity, ash, moisture, solubility, color, and freedom from extraneous materials. Odor and screen analysis must also be watched.

To secure the proper quality in each of these, it is necessary to have proper sanitation of raw materials and processing, exact temperature and pH control during precipitation and washing and automatic control during the drying process. J.C.M.

DISEASE

337. The Epidemiology of Poliomyelitis. W. Lloyd Aycock. Virus and Rickettsial Diseases, Harvard School of Pub. Health, Symposium Vol.: 555–580. 1940.

A review with blibliography. Milk-borne epidemics of poliomyelitis are discussed briefly. Transmission through milk is exceptional and plays only a small part in the epidemiology of this disease. R.E.L.B.

338. Directions for the Ascorbic Acid Therapy of Slow Breeding Bulls. P. H. Phillips. Certif. Milk, *15:* 173; 10. Sept., 1940.

Recent researches on bovine blood have shown that under certain dietary regimes cattle do not synthesize all of the vitamin C or ascorbic acid they

need for normal functions. Under such conditions the breeding efficiency of many bulls is greatly impaired. Phillips *et al.* found that subcutaneous injections of ascorbic acid cause marked recovery in 65–75 per cent of the impotent bulls which have been treated. This article describes briefly the method of treatment as an aid to the veterinary practitioner. W.S.M.

339. Types of Tubercle Bacilli in Pulmonary Tuberculosis in Northeast Scotland. STANLEY A. GRIFFITH AND J. SMITH. Lancet, *239:* 291–294. 1940.

From April, 1934, to July, 1939, 972 human cases of tuberculosis were studied and the tubercle bacilli found in the sputa identified as to type. Bovine type bacilli were found in 68 of the cases, 4 of which yielded other types as well. In the rural districts, 9.1 per cent of the cases yielded bovine type bacilli, whereas in Aberdeen only 4.4 per cent were caused by bovine types. Forty-nine of the cases caused by human type bacilli were in farmers or in persons employed on the farm, where they are liable to infect milk and milk products with tubercle bacilli. Pasteurization of milk is recommended. J.F.C.

FEEDS AND FEEDING

340. The Scientific Feeding of Animals—A War-Time Problem. A. J. AMOS AND D. W. KENT JONES. Food Manufacture, *15:* 5; 135–138. 1940.

The problem of securing proper feeding rations for farm animals in the face of war-time restrictions is a major one.

Dried brewer's grains, dried distiller's grains, dried malt culms, rice meal, bean or pea meal and even dried fruits may be used as supplements or substitutes for regular cattle feeds which are hard to obtain.

Many of the above things and in addition, locust beans, rye middlings, ripe acorns, linseed cake and others may be used for sheep, swine and poultry.

Fish meal can be replaced by a mixture of meat, bone, soybean and bean meals. J.C.M.

341. Grass Silage Steps Ahead. J. W. BARTLETT. Certif. Milk, *15:* 173; 9. Sept., 1940.

The author points out numerous problems pertaining to grass silage which still need a great amount of research. The major problems discussed in this article are: 1. Relation of moisture in grass to its successful preservation as a silage; 2. Economical methods of harvesting and silo filling, 3. The correct preservative and its application; 4. The roughage to put in the silo; 5. How much grass in the form of silage or hay shall we preserve for our livestock? W.S.M.

342. Preparation of Ammoniated Sugar Beet Pulp and Corn Silage. Use as Protein Foods for Ruminants. H. C. MILLAR. Indust. Engin. Chem., *33:* 2; 274–278. 1941.

Experiments were conducted to determine the amount of ammonia which could be fixed as water soluble nitrogen in sugar beet pulp and corn silage. When air-dry sugar beet pulp was treated with anhydrous ammonia for one hour it was changed to an attractive green or brown feed with sufficient fixed ammonia to give a product containing up to 8 per cent nitrogen. The treatment for corn silage gave a feed containing 2.34 per cent nitrogen. Most of the fixed nitrogen is water soluble. The equipment needed for ammoniating dried sugar beet pulp consists of a storage tank for ammonia, appropriate valves and measuring devices and an iron or steel ammoniation chamber. An ammoniated sugar beet pulp containing 4.4 per cent nitrogen was readily eaten by dairy cows and sheep, and by year-old dairy stock daily as offered for 3 weeks. Corn silage with increased nitrogen was readily eaten by year-old dairy stock. B.H.W.

FOOD VALUE OF DAIRY PRODUCTS

343. The Ascorbic Acid Content of Various Milk Products, Especially Dried Milk (Milk Powder). ZINNOSUKE TYO. Mitt. med. Akad. Kioto, *26:* 5; 1033–1042 (in German) (in Japanese, 1058). 1939.

The average contents of total ascorbic acid and reduced ascorbic acid, expressed in mg. per cent, in 15 samples of native dried milks were as follows: whole milk powder prepared by the spray process (4 samples) 6.34, 4.39; whole milk powder prepared by the roller process (2 samples) 5.16; 3.06; milk powder, prepared by the spray process, with added carbohydrates (4 samples) 5.18, 2.38; skim milk powder prepared by the spray process (1 sample) 8.85, 2.30; buttermilk powder (3 samples) 4.38, 1.65; lactic acid milk powder (1 sample) 7.44, 6.45, respectively. The percentage ratio of reduced to total ascorbic acids was higher in the whole milk powders than in other preparations. Both reduced ascorbic acid and dehydroascorbic acid (total-reduced ascorbic acids) of the raw milk undergo considerable destruction during the drying processes. The potentiometric pH of mixtures of 10 per cent dried milk with distilled water was 6.7–7.5 for the whole milk powders, 6.7–6.9 for the milk with added carbohydrate, and 5.2–5.5 for the buttermilk and lactic acid milk powders. The pH bore no relation to the contents of reduced and total ascorbic acids. R.E.L.B.

344. The Milk-clotting Activity of Papain. C. V. GANAPATHY AND B. N. SASTRI. Current Sc., *9:* 413. 1940.

Maleic acid, copper and H_2O_2 and other oxidizing agents destroy the milk-clotting activity of papain. Cyanide annuls the copper inhibition,

while reduction with hydrogen sulfide or cyanide restores the activity of the enzyme inactivated by the oxidizing agents. The maleic acid inhibition is a time reaction. The enzyme is irreversibly inactivated by iodoacetic acid. The press juice of pineapple, the latex of *Calatropis gigantea* and papaya latex have exactly the same milk-clotting activity. The labile sulfhydryl group is involved in this milk-clotting activity. R.E.L.B.

345. The Prevention of Hemorrhagic Disease of the Newborn by the Administration of Cow's Milk During the First Two Days of Life. LEIF SALOMONSEN. Acta Paediat., *28:* 1; 1–7. 1940.

Newborn infants commonly show an increase in coagulation time of the blood, which becomes especially marked on the third and fourth days of life. When cow's milk was fed to the newborn, starting at 2 hours of age, this prolongation in coagulation time was completely prevented in summer (August) and was considerably reduced, though not to the normal level, in winter (January-February). In infants who did not receive this treatment, hemorrhagic disease of the newborn occurred at the rate of 8.4 per thousand, while, at the same time of the year, none of the 1005 infants who were fed cow's milk on the first two days developed spontaneous hemorrhages. R.E.L.B.

346. The Gradual Evolution of Food Habits. HAZEL K. STIEBLING. Can. Dairy and Ice Cream J., *19:* 9; 48. 1940.

The author shows how environment and the availability of foodstuffs affects the diet, food habits and health of human beings. Improvement in stature and body build among immigrants to North America have been attributed to the better diets which are obtainable here. O.F.G.

347. The Vitamin G (Flavin) Content of Milk. W. H. RIDDELL. Certif. Milk, *15:* 174; 5. Oct., 1940.

This article deals with the significance of vitamin G in milk and whether or not milk is a consistently good source of this vitamin. The effect of such factors as breed, feeding, stage of lactation and so on are also discussed. W.S.M.

348. Riboflavin Determination by the Microbiological Method. AARON ARNOLD, S. T. LIPSIUS AND D. J. GREENE. Food Res., *6:* 1; 39. 1941.

The authors show that the microbiological method of Snell and Strong for determining riboflavin, which is based on the growth and acid production of *L. caseie* has a number of points to recommend it as the method of choice, particularly where a biological method is to be preferred. Its specificity was demonstrated and concordant results were obtained at three different

concentrations of the unknown, the agreement between separate samples being very satisfactory. This procedure is considerably more rapid than the rat-assay method. F.J.D.

ICE CREAM

349. Tests of Enzyme Converted Corn Sirup Reveal Desirable Qualities. P. H. Tracy and George Edman. Food Indust., *12:* 12; 43–46. 1940.

This article deals with the possibility of replacing part of the sucrose in frozen dairy products with enzyme converted corn sirup which is a new type sweetening agent containing dextrose, maltose and dextrins.

Enzyme converted corn sirup has a sweetening value two thirds that of sucrose.

Enzyme converted corn sirup depresses the freezing point more than the same amount of sucrose but less than dextrose.

There are no undesirable effects of enzyme converted corn sirup upon mix color, viscosity or melting resistance of the ice cream. It retards whipping slightly, has no effect on flavor and improves the body of the ice cream.

The use of enzyme converted corn sirup aids in prevention of surface crustation of water ices and sherbets and improves the body of these products.

A maximum sucrose replacement of one third for ice cream and one half for sherbets and ices was recommended. J.C.M.

350. Ice Cream Manufacture in War Time. L. J. Hynes. Food Manufacture, *15:* 3; 64–66. 1940.

This is a discussion of possible substitutes for the cream as a source of butterfat and for the sucrose in ice cream.

Butterfat may be obtained to some extent from reprocessed butter. Practically, though, it will be necessary to find a substitute for butterfat. Usable substitutes are refined vegetable oils, both hardened and non-hardened, which are often known as vegetable butters and the unsalted margarines of good quality.

As sucrose substitutes there are dextrose, glucose, saccharin and honey. It is not practical to substitute one or more of these for all the sucrose.

J.C.M.

351. The Inversion of Sucrose. Eric Humphriss. Food Manufacture, *15:* 6; 161–162. 1940.

As a result of the war-time scarcity of sugar the possibilities of inverting sugar and thereby increasing the sweetness for use in ice cream was investigated.

Complete inversion of sugar was attained by boiling for ten minutes with 0.10 per cent citric acid.

Taste tests indicated inverted sugar to be slightly sweeter than sucrose but when tests were made on ice cream mixes, a 9½ per cent inverted sucrose mix was always rated less sweet than an 11 per cent uninverted sucrose mix.

J.C.M.

352. **Citrus Pectates—Properties, Manufacture and Uses.** W. E. BAIER AND C. W. WILSON. J. Indust. Engin. Chem., *33:* 3; 287–291. 1941.

Two types of pectates are discussed: 1. The common non-fibrous pectate which results from the saponification of pectins; 2. A viscous, fibrous pectate which is prepared from the plant material, protopectin, by treatment with alkali. The viscous pectate may be irreversibly transformed into the non-viscous type by acid treatment. Details of production and chemical behavior of these pectates and of pectic acid are given. Many uses for pectic acid, viscous type pectate and pectate pulp are described in some detail. Mention is made of the use of the gel-forming property of viscous pectates in the manufacture of cream soups and cocoa-milk suspensions.

B.H.W.

353. **The Importance of Homogenization.** A. W. FARRALL. Ice Cream Field, *37:* 1; 19. 1941.

"Proper homogenization principally affects fat globule size and dispersion, protein stability and water binding characteristics of mix." It is further claimed that homogenization has been considered fairly satisfactory if not more than 10 per cent of the fat globules in the mix exceeded 2 microns in diameter. With the same degree of fat dispersion in two ice cream mixes, in one case accomplished by means of high pressure homogenization and in the second case brought about by relatively low homogenization pressure it is stated that protein stability is greater the lower the homogenization pressure employed. It is also claimed that for best results mixes should not be held at 160° F. for more than 30 minutes.

Fat dispersion after homogenization is classified as follows: 1. Primary dispersion—in which all globules are individually dispersed and under 2 microns in diameter; 2. Secondary dispersion—similar to (1) above except that many of the fat globules are in loose clusters; 3. Tertiary state—most of the fat globules exist in clumps which nullifies much of the work of homogenization; 4. Buttering is the fourth state—in this instance it is claimed that the fat globules have lost their original globular shape and exist in an amorphous mass.

Certain essentials are discussed, such as the necessity of the suction and discharge valves being in good condition to provide uniform steady pres-

sure, elimination of leaks, and proper installation of packing. In the conventional type homogenizer the homogenizing valve requires periodic resurfacing to insure proper operation. The author mentions a new type of single service valve developed by his company which eliminates homogenizer valve difficulties, it is stated. Directions are also given for cleaning and sterilizing homogenizers. W.C.C.

354. Vanilla Bean Situation. JOHN H. BEACH. Ice Cream Field, *37:* 1; 55. 1941.

Mention is made of the serious vanilla shortage in this country due to the effect of the war in cutting off the Madagascar supply. It is stated that because of this shortage Mexican beans are being picked earlier than normal, thereby resulting in a further decrease of 17 tons. Estimates indicate that there will be the following supply of beans from Mexico this year—200,000 pounds of whole vanilla beans and 235,000 pounds of cut beans.

Mention is made of the high tax on alcohol used in the manufacture of vanilla and other extracts. According to figures presented by the authors, the tax is from 19.32 to 24.25 times the distillers selling price of the alcohol. It is further claimed that such a condition does not exist in any other country in the world. W.C.C.

355. A Discussion of Some Factors Affecting Mix Viscosity. A. J. HAHN. Can. Dairy and Ice Cream J., *19:* 10; 64. 1940.

Mix viscosity will increase by increases in total solids, serum solids, sugar, fat and stabilizer. Use of butter or unsweetened frozen cream in the mix causes a higher viscosity than the use of fresh cream but sweetened frozen cream produces results more comparable with those obtained with fresh cream. The use of atmospheric roller powder, superheated condensed milk and sweetened condensed skim milk causes some increase in viscosity. On the addition of gelatin, viscosity increases over a period of 48 hours. Some gum stabilizers cause a rapid increase in viscosity without aging. One type of vegetable stabilizer causes immediate viscosity by reacting with calcium ions, while another type of vegetable stabilizer increases viscosity over a period of 48 hours due to slow precipitation of proteins. Calcium and magnesium ions increase viscosity whereas citrate and phosphate ions ordinarily decrease viscosity. A table is presented which shows various factors which cause changes in viscosity and the interrelation of these changes to whipping ability, body and texture and flavor. O.F.G.

356. How Can We Create New Business? C. W. ESMOND. Can. Dairy and Ice Cream J., *19:* 11, 60. 1940.

To quote the author: "What makes people buy ice cream?" is summed up in the words, "They like it." There is no sales resistance to the using

of ice cream. The author suggests increased sales of ice cream are possible by keeping the public constantly reminded of the product right at the store where it is sold, by having it attractively served and served palatably, of tirelessly reminding the public to refresh themselves. Further, get ice cream out in the open where it can be sold, not tucked away back in the stores, overwhelmed by profuse advertising of other products. O.F.G.

357. A Discussion of the Ice Cream Regulations. W. C. CAMERON. Can. Dairy and Ice Cream J., *19:* 12, 40. 1940.

This article describes changes in the regulations for ice cream in the province of Ontario. The food solids content was changed to provide that not less than 0.65 lbs. of the required 1.9 lbs. must be milk fat in the case of plain ice cream, and not less than 0.55 lbs. in the case of fruit and nut ice cream. Sherbets must include a minimum acid content of 0.35 per cent. Packages for brick and cup ice cream have been standardized as to size and are now ¼ pint, ½ pint, 1 pint, 1 quart or multiples of 1 quart. All packages must be labeled as to true volume content. O.F.G.

358. A Study of Homemade Ice Cream. V. D. FOLTZ AND W. H. MARTIN. Food Res., *6:* 31. 1941.

A study of 100 samples of homemade ice cream frozen in the tub freezer and in the mechanical freezer with and without agitation were made. The reasons mentioned for making the product were economy, 55 times; taste, 34; convenience, 25; and safety, 15.

Raw milk and cream predominated in preparing the product showing a higher average bacterial count than that found in an earlier survey of commercial samples. Incorporating all samples, refrigerator-frozen samples were bacteriologically superior to tub-frozen samples. Samples made from pasteurized milk and cream and frozen in the refrigerator compared very favorably bacteriologically with the best factory-made ice cream.

The butterfat content varied from a low of 1.4 per cent to a high of 35.4 per cent with total solids ranging from 17.84 to 53.36 per cent.

P.A.D.

359. How to Use Dextrose in Canning. E. W. EICKELBERG. Food Indust., *12:* 1; 33–35. 1940; *12:* 2; 50–51. 1940.

This gives the results of experiments on the use of dextrose in canning and the effect of various conditions on the caramelization and discoloration of the dextrose.

It was found that: 1. Time and temperature of the canning process were not necessarily causative agents of the darkening of dextrose solutions but might serve to increase the effect of other factors; 2. Metallic content, unless excessive, appeared to have little effect; 3. The influence of amino acids and

proteins on the discoloration of dextrose must be studied for each product; 4. The higher the concentration of dextrose the greater the possibility of discoloration upon processing; 5. The pH is the most important influence on caramelization in processing. As the pH decreases from neutrality to 2.5, the caramelization is inhibited and discoloration is decreased.

Fruits with a low pH and a relatively short low temperature process can be packed with 25–50 per cent of the sucrose replaced by dextrose to give a clear colored, better flavored product.

Dextrose was also found to increase shelf life of such products as citrus juices by retarding oxidation.

This indicates a possible means of enhancing the flavor of fruits and vegetables over a longer period of time. J.C.M.

360. War Time Control Troubles British Food Manufacturers. H. B. Cronshaw. Food Indust., 44–46. 1940.

This article lists some of the food manufacturer's troubles in England due to war time restriction.

The rationing of sugar affects nearly all food manufacturers. In jams the sugar content cannot be lowered appreciably without danger of spoilage; therefore, the production will have to be cut. The candy manufacturers, in general, believe that the cheaper, lower quality candy will be eliminated. The ice cream industry is busy devising means of keeping up production in the face of drastic rationing of sugar. One suggested method is by the use of sweetened condensed milk, providing this is available. J.C.M.

361. Fresh Flavor Assured by Prompt Freezing. R. E. Heyl and P. H. Tracy. Food Indust., 39–40. 1940.

In this experiment, by strict economy of time, ice cream was obtained approximately 4.25 hours after the milking period at the University barns. Ice cream was also made from mixes which were aged for 4 and 24 hours. Some of each mix was frozen in a continuous freezer and the rest in a counter freezer.

Ice cream made from the 4 and 24 hour mixes had a better body and texture, especially in the case of the counter freezer product. Aging had no effect on the flavor of the ice cream. When the ice cream was judged one week later, the ice cream frozen immediately after processing was found to retain more of its original fine flavor than the ice cream made from aged mixes. J.C.M.

MILK

362. The Production and Distribution of Milk. C. A. Wilson. Food Manufacture, *15:* 3; 71–74. 1940.

This article deals with the necessity and difficulties of producing and distributing wholesome milk in England.

It lists the responsibilities of the authorities and individuals in detail. The lack of uniformity of quality, faulty distribution and lack of proper control of the milk supply is pointed out.

As necessary improvements the author lists, among others, penalizing the unsatisfactory producer, compulsory bacteriological standard for all milk and eradication of tuberculous dairy cows. J.C.M.

363. Tests for Quality of Milk and Cream. S. T. COULTER. Nat. Butter and Cheese J., *32:* 2; 63. 1941.

For uniform results in titrating for acid, four factors must be standardized: The amount of material should be accurately measured with a 9 ml. or a 17.6 ml. pipette; 0.5 ml. of 1 per cent alcoholic solution of phenolphthalein should be used for each 9 grams of sample; dilution of sample should be limited or carefully controlled because dilution with water decreases the titratable acidity; and the end point should be attained by control of light and background during titration or by the use of color standards or both. Variations in procedure are described when titrating milk, condensed milk and cream.

Brief specific instructions for making the methylene-blue and resazurin tests are given. Two tests for mastitis are described. The strip cup, consisting of a container covered with 100 to 120-mesh wire screen, is used to collect the first streams of milk from each quarter of the cow. The presence of flakes indicate infection but not all infections will produce flakes. The test is practical for daily use on the farm but is not sensitive. The bromthymol-blue test for mastitis is described by quoting the instructions printed in New York (Geneva) Agricultural Experiment Station Bulletin 626, 1933. W.V.P.

364. Chlorine and Quality Improvement on the Farm. J. A. KEENAN. Nat. Butter and Cheese J., *32:* 2; 15. 1941.

Only proper farm methods can produce quality milk. Clean equipment, clean cows, clean milkers and prompt and proper straining and cooling are necessary. Chlorine solution is recommended as a bactericide. Two gallons of a fresh chlorine solution at each milking will serve to rinse up to 12 cans and the pails, strainers and milking machine that would be used with them. The same solution can be used for washing milkers' hands and teats and udders of the cows. If the chlorine solution is applied just before milking it will assure removal of extraneous matter and destruction of organisms on cans and utensils; as a wash for cows and for milkers' hands it prevents milk defects by removing foreign materials before it can get into milk and serves to check spread of udder troubles by machines or milkers. W.V.P.

365. The "Elwell" or Sliding Scale of Milk Prices to Consumers. E. S. ELWELL. Can. Dairy and Ice Cream J., *19:* 9; 36. 1940.

Retail home delivery of milk is breaking down rapidly because of competition from stores and milk depots. The buying public seems to expect less service and more milk for its money. Under the flat price system the cost of service is greater than that of the milk when retail home delivery is practised. The author proposes a sliding scale of prices for multiple unit buyers of milk. Under this system the one-quart consumer pays more for service per quart than does a multiple quart consumer. This plan is said to offer lower milk prices to consumers without a reduction in price to the producers. O.F.G.

366. Short-Time Pasteurization as it Looks to the Milk Plant Operator. W. D. DOTTERER. Food Indust., *12:* 2; 46–47. 1940; *12:* 3; 74–75. 1940.

This article gives the results of tests conducted on short-time pasteurization, with a holding period of 16 seconds, as compared with the accepted method, with a holding period of 30 minutes.

Milk held 16 seconds at temperatures above 158° F. gave satisfactory phosphatase test reactions but temperatures of 158° F. or lower were unsatisfactory.

Apparently short-time pasteurization was not as effective as the 30-minute holding period in killing thermoduric bacteria.

The use of a no-foam clarifier was found to have an injurious effect on the creaming ability of milk.

If very low bacteria counts are of first importance, short-time pasteurization will have difficulty in successfully competing with 30-minute holding method.

The short-time pasteurizer has the advantage of saving time and floor space, lower initial cost and a slight improvement in flavor over the 30-minute holding period. J.C.M.

NOTE: More than a year's practical experience with the short-time pasteurizer has not confirmed the experimental results on active counts. No comparisons are available on the same milk, but results from different plants indicate little if any difference in counts of thermoduric organisms. It should be noted, however, that a clarifier has been used with the short-time outfit, filters with the 30-minute holders.—W. D. DOTTERER.

367. Methods of Increasing the Viscosity of Cream. L. H. BURGWALD. Can. Dairy and Ice Cream J., *19:* 10; 60. 1940.

Pasteurization of cream reduces viscosity but is essential from the health standpoint. Homogenization of the cream increases the viscosity. A method for handling cream in vats to increase the viscosity was worked out and is as follows: Heat the cream slowly to 84° to 86° F. in a vat employ-

ing a drum type agitator which can be regulated to a speed of about 11 to 14 turns per minute. Keep about 20 to 30° F. differential between the heating medium and the cream and take about 1 hour to heat. When temperature of cream gets to 84° F., drain jacket and hold 10 minutes. Cool slowly to between 60° and 50° F. and draw off the cream in cans which are stored in ice water until the next day. This treatment increased the flow time of 20 per cent cream from 6 to 12 per cent, of 22 per cent cream from 15 to 42 per cent, and of 25 per cent cream from 18 to 56 per cent. O.F.G.

368. Minimum Pasteurization Requirements for Ice Cream. T. V. ARMSTRONG. Can. Dairy and Ice Cream J., *19:* 11; 38. 1940.

This is a review article dealing with the effect of pasteurization on microorganisms in ice cream mixes. Early work had indicated that a temperature of 145° F. for 30 minutes was sufficient for the destruction of all pathogenic organisms. Later it was found that as the sugar content increased a higher temperature was required and that efficiency of pasteurization was reduced if large numbers of heat-resistant organisms were present. Application of the phosphatase test is successful within certain limits, but sugar was found to protect phosphatase from complete inactivation at temperatures below 150° F. O.F.G.

369. Controlling the Quality of Milk and Cream. C. L. FLESHMAN. Can. Dairy and Ice Cream J., *19:* 11; 42. 1940.

The author suggests that:

1. Each plant have at least one fieldman.
2. Simple quality tests be made on incoming milk and cream.
3. Permanent quality test records be kept.
4. Each plant be in position to furnish dairy equipment and supplies to its producers.
5. Milk and cream of unsuitable quality be vigorously rejected.
6. A method of payment for milk and cream that will promote quality improvement be instituted. O.F.G.

370. Six-Day Delivery. W. F. JONES. Can. Dairy and Ice Cream J., *19:* 12; 16. 1940.

In Ottawa 6-day delivery of milk has been practiced since 1900. Among the more important points which may be affected by 6-day delivery are the following: I. Quality of products—Two deliveries are made on Saturday. Milk pasteurized on Friday is delivered on Saturday morning while milk pasteurized on Saturday morning is delivered Saturday afternoon. No apparent injury to quality occurs. 2. Service—The customer is assured of his milk supply for every day in the week. 3. Sales volume—Comparisons with other companies on 7-day delivery indicate no appreciable difference in

sales volume with the exception of extras. Extras sales seemed considerably greater with companies practicing 6-day delivery than those on 7-day delivery. 4. Controlling credit and checking books—Some slight advantage may be held by the 7-day plan. 5. Route salesmen's viewpoint—Route salesmen were 96 per cent opposed to a change from a 6-day to a 7-day delivery. 6. Sales manager's viewpoint—Advantages outweigh the 7-day system. O.F.G.

371. Effect of Roughages on the Color and Flavor of Milk. O. F. GARRETT. Certif. Milk, *16:* 178; 5. Feb., 1941.

Experiments are reported which compared the effects of various roughages on total yellow color of milk, flavor score of milk, and susceptibility of the milk to the development of oxidized flavor. The following comparisons were made: 1. Molasses alfalfa silage and spring pasture; 2. Molasses grass silage, beet pulp and citrus pulp; 3. Molasses grass silage, phosphoric acid grass silage and corn silage.

It was found that through the feeding of properly ensiled grasses and legumes, a winter milk could be produced which is superior in yellow color and flavor to ordinary winter milk produced by dry feeding or by feeding corn silage. Also, certain practices, based on practical experience, are listed and recommended for obtaining the maximum effects in producing milk of good flavor and high yellow color. W.S.M.

372. Utilization of Skim Milk in a New Potato Product. B. H. WEBB. Food Indust., *12:* 5, 48–49. 1940.

This new product may be made in the form of wafers, sticks or croutons and is made by whipping a mixture of riced boiled potatoes, skim milk, salt and pepper or other desired flavor.

The formula used is:

Peeled boiled potatoes	800	grams
Skim milk solids	80	"
Water and flavoring	200	"
Salt (pepper to taste)	15	"

A variation in overrun of 50 to 80 per cent and a specific gravity of the mix between 0.58 and 0.65 is permissible.

White potatoes of any grade that will yield a light mashed potato are suitable. Soluble spray-dried skim milk is very convenient but unsweetened condensed skim milk of 28 per cent total solids with no added water may also be used.

The proportions of skim milk and potato may be varied.

Some of the factors influencing tenderness and texture are: a too dense mix yields a hard, tough product; too rapid drying results in a hard, dense wafer, as also does the presence of fats and oils; too low drying temperature has the same result as drying too rapidly.

The dry wafer is a potato white. Toasting improves its flavor and gives it an attractive brown color.

The plain wafers had a slight cheese flavor. The addition of vegetables was tried but the flavor was not outstanding.

Packaging presented no unusual difficulties. All that was required was a moisture proof container such as cellophane or lined paper bags. J.C.M.

373. How Quick Freezing Affects Keeping Quality of Milk and Cream. C. L. ROADHOUSE AND J. L. HENDERSON. Food Indust., *12:* 6; 54–55. 1940.

An experiment was carried out using certified raw milk, pasteurized milk and cream and pasteurized homogenized milk and cream. The cans were vacuum sealed and frozen in a constant agitation freezer. The temperature of the freezing medium was – 25° F., and the cans remained in the freezing solution 23 minutes. After freezing they were placed in cases and stored at – 5° F.

The unpasteurized milks became oxidized before the end of the third week.

The bacteria counts remained low all during six months' storage and this indicated that flavor changes were chemical rather than bacterial.

It was found that there was no separation of fat globules even in the milk that was not homogenized before freezing.

After the first six weeks the flavor of all the samples changed somewhat and was considered less desirable but still acceptable for table use.

It was concluded that milk frozen by this method should have a place on long ocean voyages where fresh milk will not be available or on shorter journeys where freezing rooms are available. The advantage of this product over dried or evaporated milk is lost after several weeks.

Properly pasteurized cream frozen by this method and stored at – 5° F. was considered satisfactory for six weeks where the fresh product was not available. J.C.M.

PHYSIOLOGY

374. The Absorption of Carotene from Isolated Intestinal Loops. J. LOGAN IRVIN, JOSEPH KOPALA AND CHARLES G. JOHNSON. Am. J. Physiol., *132:* 1; 203–210. 1941.

Pure bile salts are capable of promoting the absorption of carotene from isolated intestinal loops of dogs. Sodium desoxycholate is the most effective bile salt studied but sodium cholate and sodium glycocholate are also effective.

In the presence of identical amounts of bile and lipase, the rate of absorption of carotene is greater when the initial amount of carotene in the loop

is increased. Reports on the inhibiting effect of mineral oil on the absorption of carotene are confirmed. D.L.E.

375. **The Influence of Vitamin E-Deficiency on the Endocrine Glands of Rats, Particularly on the Gonadotropic Hormone Content of the Pituitary Gland.** CLYDE BIDDULPH AND ROLAND K. MEYER. Am. J. Physiol., *132:* 1; 259–271. 1941.

The adrenal cortex and the thyroid tissue of male rats on a vitamin E-deficient diet tend to hypertrophy. At the same time the germinal epithelium of the testes is degenerating. There are no changes in the adrenals, thyroids or ovaries of female rats on a vitamin E-deficient ration.

The pituitaries of rats on an E-deficient ration also increased in weight. On assay the gonadotrophic principle of pituitaries of normal and E-deficient rats was the same but the luteinizing principle was higher in the pituitaries of E-deficient rats. D.L.E.

376. **Peroxidase Reaction. CXXXVI. Chlorine Content of the Urine of Mothers and their Arakawa Reaction.** HARUKA UMEMURA. Tohoku J. Exp. Med., 39: 2; 103–115. 1940.

The chloride retention tends to be abnormal in mothers who secrete a milk which gives a negative Arakawa reaction. R.E.L.B.

377. **Peroxidase Reaction. CXXXVII. The Influence of Emotion in Lactating Mothers on the Arakawa Reaction and the Amount and Chlorine Content of the Urine.** HARUKA UMEMURA. Tohoku J. Exp. Med., *39:* 2; 116–123. 1940.

Emotion affects the Arakawa reaction of breast milk. When the mother is worried, the Arakawa reaction of the milk becomes negative and the volume and chloride content of the urine decrease. After the source of worry has been removed and the emotional state of the mother improves, the Arakawa raction of the milk becomes positive and the volume and chloride content of the urine increases. R.E.L.B.

378. **Peroxidase Reaction. CXXXVIII. Lactose Content and Arakawa Reaction of Human Milk.** KYOSIRO SYODA. Tohoku J. Exp. Med. *39:* 2; 124–154. 1940.

The lactose content of 392 samples of milk from 200 women varied between 4.3 and 8.9 g. per 100 cc.; the average lactose content was 7.24 g. per 100 cc. Milk which gives a positive Arakawa reaction contains more lactose than one giving a negative reaction. The lactose content is slightly lower in cold weather. The highest lactose content generally occurs in the middle of the lactation period (5 to 9 months), although milk which gives a

positive Arakawa reaction contains a nearly constant amount of lactose throughout lactation. The age of the mother has no effect on the lactose content of the milk. The lactose content was higher than 7.3 g. per 100 cc. in 80 per cent of the milks with a positive Arakawa reaction, in 40 per cent of the milks with a negative Arakawa reaction and in 50 per cent of those with an intermediate reaction. Abundant milk secretion occurred in 88 per cent of the women whose milk gave a positive Arakawa reaction; as the intensity of the Arakawa reaction diminished, the percentage of cases with abundant secretion decreased. Various factors affect the lactose content of human milk. R.E.L.B.

379. Peroxidase Reaction. CXXXIX. Influence of Vitamin B_1 on the Arakawa Reaction and Chlorine Content of Human Milk. HARUKA UMEMURA. Tohoku J. Exp. Med., *39:* 2; 155–161. 1940.

The administration of vitamin B_1 to women who are secreting a milk with a negative Arakawa reaction reduces the chloride content of the milk. If the milk has a positive Arakawa reaction, its chloride content is changed little or not at all by giving vitamin B_1 to the mother. R.E.L.B.

MISCELLANEOUS

380. Keeping Out the Bacteria. PAUL S. PRICKETT AND NATHAN F. TRUE. Food Indust., *12:* 8; 34–36. 1940.

This is an article on practical methods of keeping food products "commercially sterile."

The first requirement in bacteria control is thorough washing; the second, use of a good sterilizing agent; third, elimination of dead ends and pockets that will not get properly sterilized; and fourth, making the cleaning and sterilizing of his equipment an integral part of every man's job.

Where laboratory work on the manufacturing process is needed it is available through consulting laboratories, trade associations and state experimental stations. J.C.M.

381. The Tin Situation—How It Involves the Food Manufacturer. L. V. BURTON. Food Indust., *12:* 9; 33–35. 1940.

In view of the international situation our tin supply from the Far East might be cut off. In this case we might obtain the Bolivian tin which amounts to about half our peace-time needs.

There are many substitutes for tin that are available, especially in the food industry, but these are available at an increased price. In addition to that, the use of these substitutes would create many new manufacturing problems. J.C.M.

382. New Developments in Treatment of Milk Wastes. L. F. WARRICK. Food Indust., *12:* 9; 46–48. 1940.

This article gives the results of experiments in Wisconsin on the sanitary disposal of milk plant wastes.

Some of these are: 1. Milk can and car washings; 2. Floor washings containing milk, cream, buttermilk, etc.; 3. Ice cream machine washings. Whey, skim milk, buttermilk and other strong effluents should be disposed of separately. More and more of these are being used in by-product manufacture.

It was found that the best elimination method was by means of a circular wire container of crushed rock into which the waste material is pumped at a standard rate. This container should be about 8 ft. deep.

Before the filtered waste is run into a stream it should first stand in a settling tank to remove the solids. J.C.M.

383. When is "Cleaned" Equipment Really Clean. M. E. PARKER. Food Indust., *12:* 10; 39–45. 1940.

This article gives the result of some tests by the Beatrice Creamery Company as to the benefits of acidulating the steam for sterilization of equipment.

It was found that with the regular method of washing, even when great care was used, the last equipment washed developed a bad odor if allowed to become moist. Tests revealed that mechanically cleaned cans have an inoculation of proteolytic bacteria. The protein accumulated in the wash water and formed a film on the last cans washed. This film contained the bacteria at the same time furnishing food as the film was not removed by the ordinary hot water and steam rinses and hot air blasts.

When the steam had an acid reaction the condition was corrected by releasing the film, increasing the sterilizing action of the steam, especially on bacteria capable of surviving the alkaline wash water, and by leaving the can in a slight acid condition.

Further tests with organic acids revealed that gluconic acid was the best because it did not appreciably corrode the metal and in some instances helped protect exposed iron spots. J.C.M.

384. Antiseptic and Germicidal Paints. SAMUEL S. EPSTEIN AND FOSTER DEE SNELL. J. Indust. Engin. Chem., *33:* 3, 398–401. 1941.

Literature on the subject is reviewed particularly with respect to methods of testing. Some original results are presented. Two approaches to the preparation of germicidal paints have been used: 1. The addition of antiseptic agents to otherwise standard paints and 2. Modification of the vehicle by addition of halogen to the oil. The latter is essentially chlorination of linseed oil to a 4 per cent halogen content. Method (1)

has met with little success with the exception of chlorophenols and oxyquinoline sulfate which offer some promise. Method (2) has given the best results to date but the halogenated-oil paint is far from being actually antiseptic or germicidal under all practical conditions of use. Further development work will be required before a true antiseptic or germicidal paint is obtained. B.H.W.

385. The Development of the "Frigid Food" Industry. PAUL MORTON. Can. Dairy and Ice Cream J., *19:* 9, 42. 1940.

This article describes the development of the frozen food industry and discusses obstacles encountered in packing, the problems of distribution and the education of the consumer. O.F.G.

386. Have You a Responsible "Housekeeper" in Your Plant? R. J. RAMSEY. Can. Dairy and Ice Cream J., *19:* 11, 64. 1940.

Good dairy products are associated with good housekeeping. The places to look for housekeeping defects are: 1. General appearance of property; 2. Orderliness of operations; 3. Walls and ceilings; 4. Windows; 5. Floors; 6. Odors; 7. Insect control; 8. Personnel; 9. Protection from drip and splash; 10. Sewage disposal; and 11. Plant equipment. A daily procedure for washing equipment is given. O.F.G.

387. Milk—Whither Research? J. H. SHRADER. Certif. Milk, *16:* 178; 7. Feb., 1941.

A discussion on some of the needs of the dairy industry that require recognition and treatment. Numerous research problems are suggested. W.S.M.

388. The Way to Clean Stainless Steel. G. C. KIEFER. Food Indust., *12:* 6; 48–49. 1940.

This is an article dealing with the proper methods of cleaning the 18 per cent chromium and 8 per cent nickel stainless steel.

In removing heavy accumulations of foreign matter the use of harsh abrasives or scraping tools should be avoided.

Stainless steel is not a plating. Frequent cleaning is more beneficial than harmful. For general cleaning any stainless polishing paste or powder put out by a reputable manufacturer is all right. Sometimes the use of vinegar or dilute acetic acid in the place of water increases the effectiveness of these cleaning compounds.

Sterilizing compounds should never be allowed to remain longer than six hours in continuous contact with stainless steel and a thorough washing with water should follow every exposure.

In cases where construction permits, solutions of nitric, sulphuric or hydrochloric acid can be used for removing scale or other deposits from the walls of condenser or heat exchanger tubing or brine coils. The most desirable acid solution for stainless steel is 10 to 20 per cent nitric acid but this cannot be used if ordinary steel, brass, nickel or other material soluble in this acid is present. A thorough washing with water should follow any acid cleaning, particularly with hydrochloric acid.

Removal of scale or other deposits by mechanical means should be carefully carried out. It is much safer to use small tools, manually operated, that can be conveniently handled. J.C.M.

389. What is Satisfactory Bacteriological Quality in Foods? M. E. PARKER. Food Indust., *12:* 7; 44–46, 80. 1940.

In this article the author puts forth his ideas that too much stress has been put on food sanitation and not enough on flavor.

In the field of milk, it was pointed out that the effect of working entirely in the direction of a low plate count was to produce milk with oxidized flavor and even cheese made from this milk had a poor flavor. This is especially true with the new method of plate counts using agar to which has been added lactose in order to get a more nearly correct count of lactic acid bacteria.

J.C.M.

390. Ultraviolet Finds New Applications. T. R. PORTER. Food Indust., *12:* 7; 51–53. 1940.

Germicidal ultraviolet irradiation by the use of special lamps has found new and wider use in meat, baking, fish, dairy and other industries.

As applied to the dairy industry, these lamps have been of value.

J.C.M.

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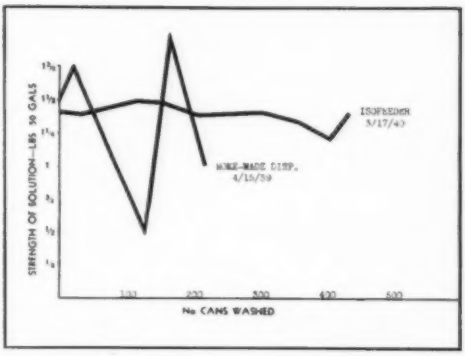

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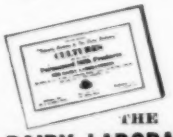

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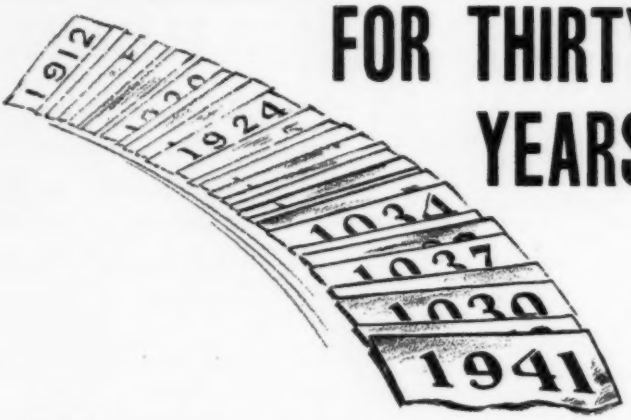

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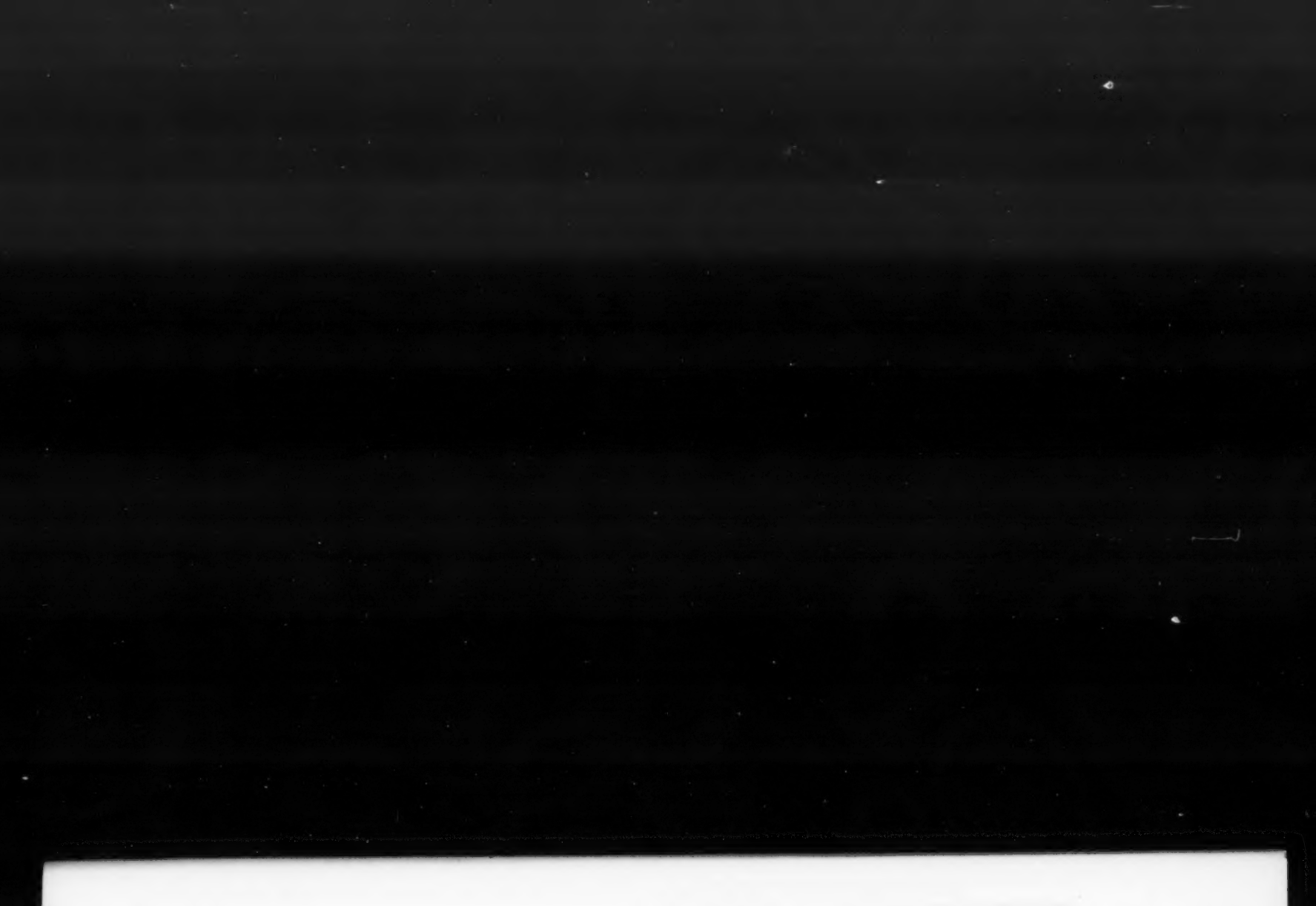

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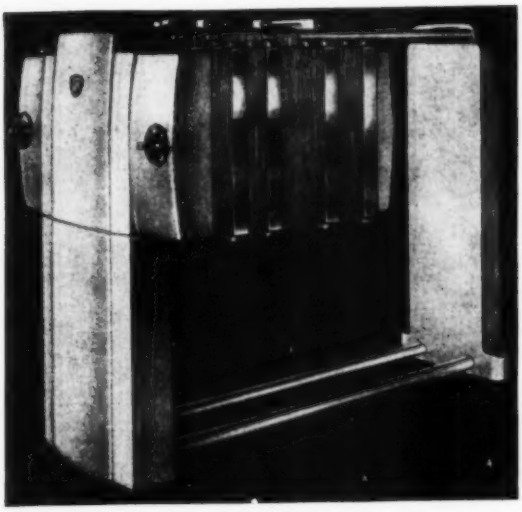

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